Neurocognitive mechanisms and vulnerability to autism and ADHD symptoms in the 22q11.2 deletion syndrome

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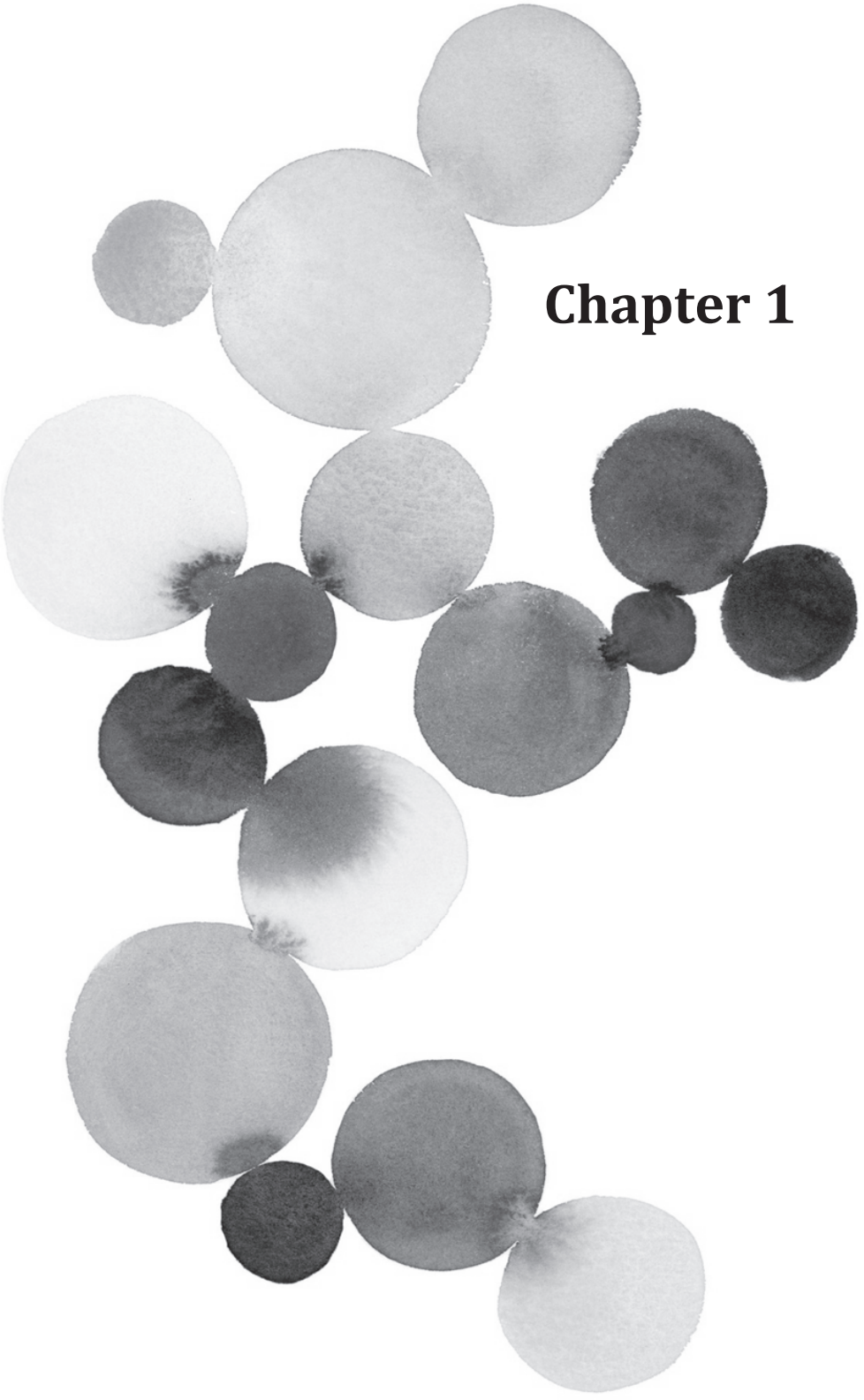

Chapter 1

General Introduction

Neurodevelopmental disorders

Neurodevelopmental disorders have an onset in early childhood. The origin, expression and developmental trajectories of these disorders is determined by genetic factors, often in interaction with the environment. For some of these disorders it is suggested that they share at least part of their genetic etiology (Rommelse *et al.* 2010; American Psychiatric Association. 2013; Posthuma & Polderman, 2013; McCarthy *et al*. 2014). Children diagnosed with these disorders experience the impact of associated developmental difficulties in their personal, social, academic, and occupational functioning during lifetime. These deficits include specific problems with learning, executive functioning, or more global impairments of social skills or intelligence (American Psychiatric Association. 2013). Discovering the mechanisms involved in the outcomes of these disorders is important to improve the developmental perspectives of these children. It is recognized that the number of symptoms accompanying these disorders and its severity can differ across individuals. This, so called, variable expressivity of symptoms might be an important starting point in studying the mechanisms that determine severity of symptomatology and developmental outcomes of these disorders (Reynolds & Mayfield, 2011).

Two neurodevelopmental disorders of which it is widely known that genetic factors are involved and for which the high frequency of comorbid occurrence suggests an overlap in genetic etiology are autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder (ADHD) (Ronald *et al.* 2008; Rommelse *et al.* 2010; Vorstman & Ophoff, 2013). Variable expressivity plays a role in both disorders and is also recognized by the Diagnostic Statistical Manual, 5th edition (DSM 5) through the inclusion of specifying severity of present symptoms. Diagnostic criteria of ASD include persistent deficits in social communication and social interaction as well as restricted, repetitive patterns of behavior, interest and activity. ADHD is characterized by severe symptoms of inattention, hyperactivity and impulsivity associated with cognitive and behavioral problems that interfere with daily functioning and development (American Psychiatric Association. 2013). Because of the suggested overlap in genetic etiology of ADHD and ASD, investigating a genetic syndrome that is associated with symptoms of both disorders is a unique opportunity to improve our knowledge about these disorders (Rutter 1997; Scourfield *et al.* 1999).

The 22q11.2 deletion syndrome is an example of a genetic disorder that is known to be associated with ASD and ADHD. Investigating neurocognitive dysfunctions as possible underlying mechanisms of the behavioral and emotional problems of these disorders in 22q11DS may provide insights in the etiology of ASD and ADHD and enlarge our knowledge about gene-brain-behavior relationships.

The 22q11.2 deletion syndrome

The 22q11.2 deletion syndrome (22q11DS), also known as the velo-cardio-facial or DiGeorge syndrome is one of the most commonly known recurrent copy number variants (CNVs) associated with a high vulnerability to psychopathology. The prevalence of 22q11DS is estimated around 1: 2,000-4,000 live births, with boys and girls equally affected. Each year around 50 children are born in the Netherlands with this syndrome (Devriendt *et al* 1998; Oskarsdottir *et al.* 2004; Shprintzen 2008). This autosomal dominant genetic disorder is caused by a microdeletion on the long arm of chromosome 22. The inherence rate of the syndrome is 50%, however in 90% of the cases the deletion is a 'de novo' mutation where none of the parents carry the genetic defect. The phenotypic expression of the syndrome is characterized by a diverse variability of physical, metabolic, endocrine and behavioral features (Bassett *et al.* 2011). Physical manifestations include conotruncal cardiac anomalies, palatal anomalies, nasal regurgitation, and/or hypernasal speech, immunodeficiency, hypocalcemia and typical facial features (Swillen *et al.* 2000; Green *et al.* 2009; Bassett *et al.* 2011; Cancrini *et al.* 2014). The neurocognitive phenotype is characterized by delays in motor development and speech, and language difficulties. Learning difficulties are common and most individuals with 22q11DS function at an intellectual level of borderline or mild to moderate intellectual disability (De Smedt *et al.* 2007; Niklasson & Gillberg 2010; Philip & Bassett 2011; Duijff *et al.* 2012). The behavioral phenotype of the syndrome is highly variable, including ADHD, ASD, anxiety disorders, oppositional deficit disorder, and mood disorders (Jolin *et al.* 2009; Baker & Vorstman 2012; Jonas *et al.* 2014; Schneider *et al.* 2014). Around 25% of the patients with the syndrome develop schizophrenia in adolescence or adulthood (Murphy *et al.* 1999; Schneider *et al.* 2014).

Over the last years a large amount of studies investigated the cognitive and the behavioral phenotype of 22q11DS providing insights in the high variability of these phenotypes (Philip & Bassett 2011). Knowledge about the association between the cognitive and behavioral phenotype may lead to a better understanding of the developmental pathways of the syndrome and improve interventions and treatment strategies. However, only a limited number of studies investigated the association between cognitive functioning and the development of emotional and behavioral problems. The findings thus far are inconsistent, with some studies failing to find an association between degree of cognitive impairment and psychopathology (Janssen *et al.* 2007; Hooper *et al.* 2013; Niarchou *et al.* 2014), while in other studies differences in neurocognitive profiles have been reported between individuals with and without psychopathology (Chow *et al.* 2006; Hooper *et al.* 2013). Differences in neurocognitive functioning were found between adults with 22q11DS with and without schizophrenia, despite the finding that mean estimated IQ levels did not differ (Van Amelsvoort *et al.* 2004;Chow *et al.* 2006). In adolescents, lower full scale IQ in childhood was found predictive for the severity of schizophrenia symptoms (Hooper *et al.* 2013). Most of these studies focused on the mechanisms involved in the emergence and severity of (prodromal) symptoms of schizophrenia in patients with 22q11DS. To understand the association between the genotype and phenotype

of the syndrome, it is important to find out if and how cognitive dysfunctions of children with 22q11DS are involved in the emergence and severity of associated social and behavioral outcomes (Shprintzen 2000).

Neurocognitive functions and autism and ADHD symptomatology

There are different ways to investigate how genetic factors, in interaction with the environment, influence brain development and function as well as the behavioral outcomes that are ultimately associated with it. One approach is to look at associations between disabilities at the behavioral level and disturbances in functioning of the developing brain; the neuropsychological perspective (Goldstein & Reynolds 2011; Swaab *et al.* 2011). Neurocognitive functions are used to process information and direct behavior as an intention to influence, or a response to the outside world. These functions can therefore be seen as an expression of the complex mechanisms in the brain and are associated to specific areas or networks in the brain (Swaab *et al.* 2011). Neurocognitive functions as reflection of brain functioning are useful for entangling the associations between genetic factors and social and behavioral problems associated with ASD and ADHD. Studying the association between behavior and neurocognitive processes in children and adolescents with 22q11DS may help to clarify the association between a genetic factor (22q11DS) and the development of social and behavioral problems through the mediating role of these neurocognitive dysfunctions. The high prevalence of autism and ADHD symptomatology in children and adolescents with 22q11DS makes this syndrome highly relevant in investigating the mechanisms on a neurocognitive level that possibly underlie the behavioral and emotional problems that are characteristic for autism and ADHD. Additionally, knowledge about the specificity of impairments in cognitive functioning and its relations to vulnerability to autism and ADHD symptoms may help develop interventions or adjust treatments to the needs of these children. This knowledge may bring us further in understanding developmental trajectories of ASD and ADHD, especially in individuals with 22q11DS.

Objective of the current thesis

The objective of this thesis is to understand the mechanisms that result in vulnerability to autism and ADHD symptomatology in individuals with 22q11DS. To this purpose we focused on the associations between neurocognitive functioning and symptomatology of the neurodevelopmental disorders ASD and ADHD.

Participants and instruments

The studies reported in this thesis are part of a nationwide study and include 102 children and adolescents with 22q11DS aged 9 – 18.5 years at time of assessment. Intellectual functioning was assessed using the Wechsler Intelligence Scales (Wechsler 1974; 2002; 2005a; 2005b). Various executive functions were evaluated using the Amsterdam Neuropsychological Tasks (ANT) program (De Sonneville 1999; 2005), the Wisconsin Card Sorting Test (WCST, Heaton et al. 1993) and the Rey-Osterrieth Complex Figure (RCFT, Rey 1964). Visual (social) information processing was assessed with the use of the ANT program (De Sonneville 1999; 2005). Detailed descriptions of tasks and procedures are provided in the respective chapters.

The described variability in expression of ASD and ADHD and the clinical reality of co-occurrence of ASD and ADHD symptomatology in 22q11DS encouraged us to investigate the severity of the associated social and behavioral problems. To this end, we investigated the three major domains of ADHD symptomatology: inattention, hyperactivity, impulsivity. Symptoms were rated with a semi-structured interview based on the criteria of the DSM-IV as a measure of severity of ADHD symptoms. The interview consisted of items comparable to those of the CRS-R (Conners 1997) and the Dutch version of the ADHD DSM-IV rating scale (Kooij *et al.* 2008). To investigate severity of symptoms on the three major domains of autism symptomatology: reciprocal social interaction, communication impairment, and repetitive and stereotyped behaviors, the algorithmic scores of the Autism Diagnostic Interview-Revised were used (Rutter *et al.* 2003).

Outline

For a thorough investigation of how neurocognitive processes are associated with the severity of autism and ADHD symptomatology in patients with 22q11DS the following topics were addressed:

1) In idiopathic ASD and ADHD populations specific impairments in subdomains of intelligence have been found, but in 22q11DS only few studies focused on factor and subtest levels of intelligence and no consistent associations have been reported yet. The study presented in chapter 2 aimed to add to literature thus far by expanding the knowledge about the association between profiles of intelligence and the neurodevelopmental disorders ASD and ADHD. Therefore, we assessed intellectual functioning on global and subdomain levels in the total sample (N=102) and explored the associations between strengths and weaknesses in intelligence profiles and the severity of symptomatology of both disorders (Chapter 2).
2) To explore whether a specific profile of (dys)executive functions can be found in individuals with 22q11DS, which is possibly associated with the social and behavioral problems that are part of ASD and ADHD, a wide range of executive functions was evaluated in a subsample of 58 individuals with 22q11DS. Associations between the quality of executive functioning and the severity of autism and ADHD symptoms were investigated (Chapter 3). For both ADHD and ASD it is known that deficits in executive functions underlie

behavior and adaptation problems that are part of these disorders. Investigating if, and how these deficits in executive functions are associated with the severity of these problems in individuals with 22q11DS may enlarge our knowledge about the underlying mechanisms of the neurodevelopmental disorders ASD and ADHD and provide opportunities to develop cognitive interventions.

3) Social problems are part of the core problems in both ADHD and ASD and social cognitive skills contribute to the development of adequate social behavior. Therefore in a subsample of 45 individuals it was investigated how quality of social cognitive functioning is associated with the severity of social behavioral problems in 22q11DS (Chapter 4). Based on previous findings of deficits in the processing of visuospatial information, we included two tasks that examine quality of face recognition and facial emotion recognition, respectively, and a pattern recognition task measuring quality of abstract visuospatial information processing as a contrast.
4) Because it is known that social deficits are part of the phenotypic expression of 22q11DS and associations have been found between COMT and plasma proline and social cognition, in the final study we focused on the association between the genotype of the remaining allele of COMT, and plasma levels of the amino acid proline, and the high vulnerability to social cognitive and behavioral deficits in the same subsample of 45 individuals with 22q11DS (Chapter 5).

A summary and integrated discussion of the presented finding will be provided in the final chapter (Chapter 6).

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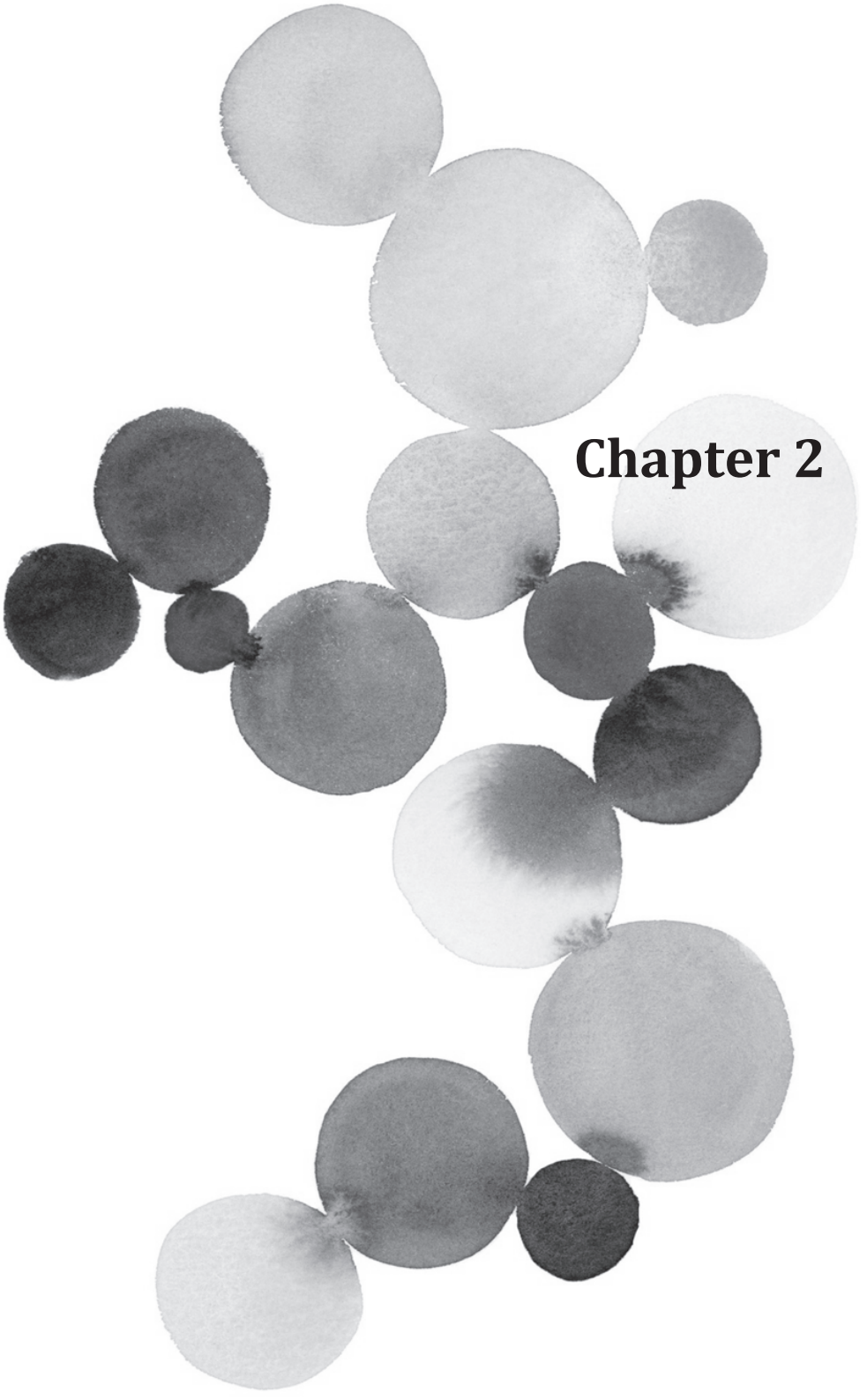

Chapter 2

Intellectual functioning in relation to autism and ADHD symptomatology in children and adolescents with 22q11.2 Deletion Syndrome

Hidding, E., Swaab, H., de Sonneville, L. M. J., van Engeland, H., Sijmens-Morcus, M. E. J., Klaassen, P. W. J., Duijff, S. N., & Vorstman, J. A. S. (2015). Intellectual functioning in relation to autism and ADHD symptomatology in children and adolescents with 22q11.2 deletion syndrome. *Journal of Intellectual Disability Research*. doi:10.1111/jir.12187

Abstract

The 22q11.2 deletion syndrome (22q11DS; velo-cardio-facial syndrome) is associated with an increased risk of various disorders, including autism spectrum disorder (ASD) and attention-deficit-hyperactivity disorder (ADHD). With this study we aimed to investigate the relation between intellectual functioning and severity of ASD and ADHD symptomatology in 22q11DS.

A sample of 102 individuals (62 females) with 22q11DS aged 9 to 18.5 years was assessed using age appropriate Wechsler scales of intelligence as well as psychological and psychiatric assessment to evaluate the presence of ASD and ADHD symptomatology.

Intelligence profiles were characterized by lower scores on the factor Perceptual Organization and higher scores on the factor Processing Speed, with on subtest level higher scores on Digit Span and lower scores on Arithmetic and Vocabulary as compared to the mean factor or subtest score respectively. No differences in intelligence profiles were found between subgroups with and without ASD and/or ADHD. Low scores on Coding were associated with higher severity of ASD symptomatology, while lower scores on Block Design were associated with more severe ADHD symptomatology.

On several subdomains of intelligence poorer performance was associated with higher severity of ASD and ADHD symptomatology. The impact of developmental disorders in 22q11DS can be traced in specific domains of intellectual functioning as well as in severity of symptomatology.

Introduction

The 22q11.2 deletion syndrome (22q11DS), also known as velo-cardio-facial syndrome (VCFS), is a congenital syndrome with an estimated incidence of 1 in 4000 live births (Devriendt *et al*. 1998; Oskarsdottir *et al*. 2004; Shprintzen 2008). The 22q11DS can be considered as a genetic disorder associated with altered development of the brain (Antshel *et al*. 2008). The clinical phenotype during childhood includes lower intelligence and higher vulnerability to symptomatology of specific disorders, such as autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD) (Antshel *et al*. 2005b; Gothelf *et al* 2008; Meechan *et al*. 2011; Philip & Bassett 2011; Vorstman *et al*. 2006). There is robust evidence for an important role of hereditary factors in the etiology of ASD and ADHD in the general population (Faraone *et al*. 2005; Ronald & Hoekstra 2011; Freitag 2007; Rommelse et al. 2010). As particular cognitive profiles have been found in idiopathic ASD and ADHD, one might question whether specific cognitive characteristics are associated with higher vulnerability to develop ASD and ADHD symptomatology in 22q11DS as well. Therefore, analysis of the relation between these symptoms and intellectual functioning on global and subdomain levels might help to further understand the dynamics of developmental vulnerability in 22q11DS.

In both ASD and ADHD, profiles of the Wechsler Scales according to the factors of Kaufman (1981) are characterized by low scores on the factors Freedom from Distractibility (reflecting attention and short term memory) and Processing Speed (reflecting visual information processing and visual memory) as compared to scores on Verbal Comprehension (reflecting verbal knowledge and use of verbal skills) and Perceptual Organization (reflecting non-verbal reasoning and visual spatial organization). Looking at the level of subtest performance groups of children with ASD and ADHD both are found to show fewer difficulties on a visual-motor subtest (Symbol Search) that requires constant shifting between symbols as compared to a subtest (Coding) in which the symbols are fixed, or, alternatively, they show poorer perceptual motor integration in more complex tasks (Calhoun & Mayes 2005; Oliveras-Rentas et al. 2012; Sattler 2001). Characteristically, children with ASD show more difficulties with reasoning when a social component is incorporated (Comprehension) as compared to reasoning apart from the social context (Block Design: Calhoun & Mayes 2005; Oliveras-Rentas *et al*. 2012; Sattler 2001). Further, communication abilities in children with ASD appear to be associated with speed in visual motor integration (Coding) and perceptual discrimination (Symbol Search). Also more difficulties with social interaction are associated with lower verbal learning ability (Vocabulary) and poorer verbal social judgment (Comprehension). These findings indicate that specific impairments in subdomains of intelligence and ASD symptomatology are associated, probably reflecting the impact of cognitive weaknesses increasing the risk for higher levels of ASD symptoms (Oliveras-Rentas *et al.* 2012).

Despite these findings in idiopathic ASD and ADHD populations, in 22q11DS research only limited attention has been given to the relation between ASD or ADHD symptoms and domains of intellectual functioning. This might be due to the fact that in the 22q11DS population intelligence is found to be highly variable as reflected in

mean full scale intelligence scores (FSIQ) ranging from moderate intellectual disability to borderline or even average scores (De Smedt *et al*. 2007; Moss *et al*. 1999; Niklasson & Gillberg 2010; Swillen *et al*. 1997; Duijff *et al*. 2012). The verbal domain (VIQ) of intellectual functioning is often found to be better developed as compared to the performance domain (PIQ) (De Smedt *et al*. 2007; Jacobson *et al*. 2010; Moss *et al*. 1999; Swillen *et al.* 1997). However, other studies found reversed differences or reported profiles without significant differences between scales (Campbell *et al*. 2009; Lewandowski *et al*. 2007). The variability in intelligence level and inconsistency in VIQ-PIQ discrepancies in 22q11DS may complicate the search for a relation between intellectual functioning and vulnerability to symptoms of developmental disorders. Using the Kaufman factors, Moss *et al.* (1999) found a significant discrepancy with higher scores on Verbal Comprehension as compared to Perceptual Organization in participants with 22q11DS that was larger than the more global VIQ-PIQ discrepancy. In addition to these findings on factor levels of intelligence, research also indicated significant variability within the subtest profile of the Wechsler Intelligence Scales (Moss *et al.* 1999; Duijff *et al.* 2012). Most studies investigating intelligence in 22q11DS in relation to ASD and ADHD symptomatology did not find a relation between FSIQ level and ASD symptoms (Vorstman *et al*. 2006), or between FSIQ, VIQ or PIQ and ADHD symptoms, respectively (Gothelf *et al*. 2007; Green *et al*. 2009; Hooper *et al*. 2013). By contrast, the group of Niklasson and Gillberg found higher levels of intelligence to be associated with lower levels of ADHD and ASD symptomatology (Niklasson *et al*. 2005). However, after extending their cohort (n=100) they could not replicate this finding, although they reported differences in subtest profiles within the factors Verbal Comprehension, Freedom from Distractibility and Processing Speed between groups with and without ADHD or ASD (Niklasson & Gillberg 2010).

Hence, studies so far did not find consistent profiles in 22q11DS and rarely focused on factor and subtest levels of intelligence in relation to developmental disorders, although other studies suggest that such consistent profiles of intelligence exist in clinical groups with idiopathic ASD and ADHD (Calhoun & Mayes 2005; Oliveras-Rentas *et al*. 2012). Therefore, we set out to investigate the relation between profiles of intelligence and severity of ASD and ADHD symptomatology. To understand the mechanisms that result in vulnerability to ASD and ADHD, it has been argued that the use of categorical diagnostic systems to identify these disorders is inadequate in 22q11DS because individuals with the syndrome often appear to meet the criteria for multiple diagnoses simultaneously (Baker & Vorstman 2012). Therefore, a focus on symptom severity instead of diagnoses seems justified.

Based on the results of Niklasson and Gillberg (2010), we hypothesized that we would find relations between specific subtests of intelligence and severity of ADHD or ASD symptomatology in 22q11DS. In their study, however, ASD and ADHD were analyzed categorically and results were grouped. Because of the evidence for different intelligence profiles in idiopathic ADHD and ASD groups, we hope to expand knowledge about these relations by focusing on severity of ASD and ADHD symptomatology separately. Supported by findings in children diagnosed with these disorders in the general population, we hypothesized shifting abilities and poorer perceptual motor skills to be related to more severe ASD and ADHD symptomatology

(Calhoun & Mayes 2005; Oliveras-Rentas *et al.* 2012). Further, difficulties with social reasoning (as measured with the subtest Comprehension) are expected to be related to more severe ASD symptoms only. Given the evidence in 22q11DS of more deficits in cognitive functioning in males versus females (Antshel *et al*. 2005a; Niklasson & Gillberg 2010) and the higher prevalence and severity of ASD and ADHD symptoms in males (Novik *et al*. 2006; Werling & Geschwind 2013; American Psychiatric Association 2013), we also expected more severe impairments in males as reflected by lower scores on domains of intellectual functioning and more severe ASD and ADHD symptomatology.

Method

Recruitment

This study was part of a nationwide study and included 102 children and adolescents, (inclusion criterion age 9-20 yr.), with 22q11 Deletion Syndrome, as confirmed with a fluorescence in situ hybridization. Participants were recruited through the Department of Child and Adolescent Psychiatry of the University Medical Center Utrecht as well as from the 22q11DS parents' network in the Netherlands by posting a request on the network's newsletter and website. When an application for participation was received, parents and participants were informed and received a complete description of the study in writing before they decided on participation. Written informed consent was obtained from participants and parents or caretakers. The assessment protocol is part of a larger ongoing longitudinal behavioral and genetic study on 22q11DS that has been approved by the Dutch Central Committee on Research Involving Human Subjects . Assessments took place at the outpatient center of the University Medical Center and were carried out under supervision of an experienced child psychiatrist and child neuropsychologist.

Sample

In the present study, 62 female and 40 males with 22q11DS participated (mean age 13.2, SD=2.6, range 9-18.5). Females had significant higher FSIQ compared to males. In a previous study the FSIQ data of 60 of these participants were reported in relation to psychiatric symptoms (Vorstman *et al*. 2006). In the current study the dataset was extended to n=102, while the analyses were expanded including a thorough investigation of intelligence on factor and subtest level and by focusing on severity of ASD and ADHD symptomatology separately.

Measures

Psychiatric classifications were made according to DSM-IV criteria, resulting from a multidisciplinary consensus meeting headed by an experienced child psychiatrist, on the basis of clinical structured and semi-structured interviews (with both the child and the caregivers), observations of the child and questionnaires, and intelligence assessments.

The assessment protocol included *the Autism Diagnostic Interview-Revised* (ADI-R*;* Rutter *et al*. 2003), scored by certified interviewers, used to quantify autistic symptoms. The ADI-R provided scores for the three domains in which children with ASD experience difficulties (reciprocal social interaction, communication impairment, repetitive and stereotyped behaviors). The classifications of autism and pervasive developmental disorder not otherwise specified are referred to as ASD. In addition the *Schedule for Affective Disorders and Schizophrenia for School-Age-Children-Present and Lifetime* Version (K-SADS-PL; Kaufman *et al*. 1997) was used to quantify mood disorders and psychotic symptoms.
Furthermore, information from the caregivers and the teachers was obtained using the Child Behavior Checklist, the Teacher Rating Form (CBCL 6-18, TRF 6-18; Achenbach 1991, Achenbach & Rescorla 2001) and Conners' Rating Scales-Revised (CRS-R; Conners 1997). Table 1 provides an overview of the formal psychiatric classifications of the sample, reflecting the multidisciplinary clinical consensus based on all available patient information.

Severity of ASD and ADHD symptomatology

In some cases, the formal diagnoses deviate from the classifications that would be obtained if only the outcomes of the questionnaires were used. The DSM-IV guidelines do not allow to diagnose both ADHD and ASD in one individual (American Psychiatric Association 2000), as a result, a formal diagnosis of ADHD was only made in four individuals (Table 1). In those cases in which the ASD symptomatology was more dominantly present explaining also the ADHD symptoms, no (additional) ADHD diagnoses was made based on such present symptoms. Two individuals were diagnosed with ADHD comorbid to an ASD diagnosis because this ASD diagnosis could not explain the severely present comorbid ADHD symptomatology (Table 1). Because of the high prevalence of both ASD and ADHD in 22q11DS the possible co-occurrence of symptoms of both disorders was also investigated. To this end, we allocated the diagnosis ADHD to any subject who passed six or more items in any of the three ADHD domains (inattention, hyperactivity, impulsivity) as rated with a semi-structured interview based on the criteria of DSM-IV. This interview consisted of comparable items as the CRS-R (Conners 1997) and the Dutch version of the ADHD DSM-IV rating scale (Kooij *et al*. 2004). Likewise, the diagnosis ASD was assigned in accordance to the ADI-R score. Table 2 provides the distribution of ASD, ADHD and ASD comorbid ADHD, other comorbidity not included. The sum scores of the items of the three ADHD domains as rated with the structured interview were used as a measure of severity of ADHD symptoms. The algorithmic scores of the three domains of the ADI-R were used as a measure of autism symptoms.

Intellectual functioning

Intellectual functioning was assessed, using the Wechsler Intelligence Scales for Children WISC-III (Wechsler 2002, Wechsler 2005b). In three cases the former version WISC-R was used (Wechsler 1974), in eight cases the adult scale (WAIS-III; Wechsler 2005a) for adolescents older than 16 years was used. According to the Kaufman factor structure and validity research of the Dutch Wechsler Intelligence scales (Wechsler 2002), factors were defined as follows: *Processing Speed (PS)*,

including the subtests Symbol search and Coding (WAIS-III: Digit symbol coding and Symbol Search), *Verbal Comprehension (VC)*, composed by the four subtests Information, Similarities, Vocabulary, and Comprehension (WAIS-III: Information, Similarities, Vocabulary), reflecting a verbal component of intelligence excluding the more mathematical tests and tests that ask for working memory and processing speed, and *Perceptual Organization (PO)*, composed by the four subtests Picture Completion, Picture Arrangement, Block Design, and Object Assembly (WAIS-III: Block Design, Matrix Reasoning, Picture Completion). Research comparing performances on the WAIS-III and WISC-III for a group of 16-year-olds found high correlations between performances of those group on both tests on the factors (Groth-Marnat, 2003; VC =.87, PO =.74, PS =.79).

Statistical analyses

Differences in intelligence profiles of children with 22q11DS with and without ASD and/or ADHD were tested using General Linear Model (GLM) – mixed models with *Factor* (PS, PO, VC) and *Subtest* (Information, Similarities, Arithmetic, Vocabulary, Comprehension, Digit Span, Picture Completion, Coding, Picture Arrangement, Block Design, Object Assembly, Symbol Search) as within subject (WS) factors, respectively, and *Group* (no ASD or ADHD, ASD and ADHD, and ASD only) as between subjects factor. The subtests Matrix Reasoning and Letter-Number Sequencing of the WAIS-III were not included because data of only eight participants were available. To bring out relative strengths and weaknesses in intelligence factor and subtest profiles, deviation contrasts, comparing scores with the overall mean subtest or factor score of each group, were used. Because the ADHD-only group consisted of eight participants, this group was excluded from analyses. Prior to analysis, normality of the data was examined and confirmed using Shapiro-Wilk tests (α=.01).
To examine the relation between intelligence and severity of ADHD and ASD symptomatology, multiple regression analyses were performed. Pearson correlations were calculated first, to determine whether age and gender were related with severity of autistic or ADHD symptoms. Only gender appeared to be correlated with symptom severity. Next, partial correlations were computed of intelligence factor and subtest scores with severity of autistic and ADHD symptoms, controlling for gender (small effect size: r = 0.1-0.23; medium: r = 0.24-0.36; large: $r \geq 0.37$; Cohen 1992).
Subsequently, factors or subtests of intelligence that were significantly correlated ($p \leq .05$, 1-tailed) with ASD or ADHD severity were planned to be included in the first step of the regression analyses. Additionally, the variable gender, when significantly correlated with the outcome variable, was entered in the second step of the regression analyses.
Moderation and mediation analyses were performed using methods of Baron & Kenny (1986) and Aiken & West (1991).

Table 1. ***Psychiatric classifications according to DSM-IV criteria with primary diagnoses and comorbid diagnoses.***

Diagnostic classification (primary)		**Comorbid diagnoses****				
	N	ASD	ADHD	Dep.dis	ODD*	Psych.dis
Autism spectrum disorder (ASD)	49		2	8	1	7
Attention Deficit Hyperactivity Disorder	4	1			1	1
Anxiety Disorder	3					
Conversion Disorder	1					
Depressive disorder (Dep.dis)	2					
Psychotic disorder (Psych.dis)	2					
Without psychiatric classification	41					
Total	102	1	2	8	2	8

* Oppositional defiant disorder
** Represent comorbid diagnoses within the total N of 102

Table 2. ***Distribution of groups with and without ASD, ADHD, and ASD comorbid ADHD*.***

Classification	*N*
No ASD or ADHD	44
Comorbid ASD and ADHD	16
ADHD only	8
ASD only	34
Total	102

*based on ADI-R score and the ADHD score of the DSM-IV structured questionnaire

Results

Comparisons on factor level

A significant WS effect of Factor was found [$F(2,164)=8.349$, $p<.001$, $\eta_p^2=.092$], but no main effect of Group ($p=.662$). The deviation contrast showed that performance on the factor PO was significantly poorer [$F(1,82)=13.681$, $p<.001$, $\eta_p^2=.143$] than the mean performance of the three factor scores ($M=72.19$), while performance on PS was significantly better [$F(1,82)=13.794$, $p<.001$, $\eta_p^2=.144$] (Table 3). The interaction between Factor and Group was not significant ($p=.327$), indicating that the factor profile did not differentiate between the groups.

Comparisons on subtest level

A significant WS effect of Subtest was found [$F(11,858)=9.748$, $p<.0001$, $\eta_p^2=.111$], but no main effect of Group ($p=.620$). The deviation contrast revealed better performances on Digit Span [$F(1,78)=47.690$, $p<.0001$, $\eta_p^2=.379$] as compared to the mean subtest score ($M=5.2$) and poorer performances on Arithmetic [$F(1,78)=51.281$, $p<.0001$, $\eta_p^2=.397$] and Vocabulary [$F(1,78)=21.411$, $p<.0001$, $\eta_p^2=.215$] (Table 3). The interaction between Subtest and Group was not significant ($p=.153$), indicating that the subtest profile did not differentiate between the groups.

Table 3. ***Intelligence profiles of subjects with and without ASD/ADHD[1].***

	No ASD or ADHD			**ASD and ADHD**			**ASD only**			**Total**		
	N	*M*	*SD*	*N*	*M*	*SD*	*N*	*M*	*SD*	*N*	M	*SD*
TIQ	39	67.8	13.6	16	64.7	12.5	34	65.6	11.8	89	66.4	12.6
VIQ	38	71.9	14.9	16	69.5	13.2	34	67.9	13.0	88	69.9	13.8
PIQ	38	69.6	12.9	16	65.1	12.8	34	67.5	12.7	88	67.9	12.8
VCF	38	73.1	15.1	16	72.6	15.1	34	69.2	14.0	85	71.5	14.7
POF	39	70.1	12.6	16	66.8	14.6	34	70.4	12.8	85	69.8*	12.9
PSF	37	77.7	13.9	15	74.9	15.2	33	72.8	14.3	85	75.3*	14.3
Information	39	5.2	3.5	16	5.1	2.9	34	4.8	2.8	81	4.9	3.1
Similarities	39	5.6	3.2	16	5.6	3.6	34	5.5	3.1	81	5.5	3.1
Arithmetic	39	4.6	2.8	16	2.8	2.1	34	4.2	2.2	81	4.1*	2.5
Vocabulary	39	4.6	2.9	16	4.4	2.7	34	3.5	2.6	81	4.2*	2.8
Comprehension	39	4.8	2.9	16	5.7	3.5	34	4.5	3.5	81	4.9	3.2
Digit Span	39	7.4	3.4	16	6.6	2.9	34	6.4	2.9	81	6.9*	3.1
Picture Completion	39	5.3	2.6	16	4.5	2.9	34	5.2	3.2	81	4.9	2.7
Coding	39	6.1	2.7	16	5.1	3.0	34	4.9	2.9	81	5.6	2.8
Picture Arrangement	39	5.0	2.7	16	4.8	3.2	34	5.0	3.0	81	5.1	3.0
Block Design	39	5.1	2.6	16	4.1	2.5	34	5.3	2.7	81	4.9	2.7
Object Assembly	39	5.5	2.9	16	4.7	3.4	34	5.7	3.1	81	5.5	3.1
Symbol Search	37	5.7	3.4	15	5.7	3.3	33	4.8	3.2	81	5.4	3.3

*≤.001 when compared to factor mean and subtest mean, respectively
[1]based on ADI-R score and the ADHD score of the DSM-IV structured questionnaire

Severity of autism symptomatology in relation to intelligence

Both the ADI-total score and the ADI-reciprocal social interaction score were negatively correlated with the factor VC, reflecting more severe autism symptomatology to be related to lower scores on VC. No other correlations were found between any of the Kaufman factors and autism symptomatology (Table 4). ADI-total correlated negatively with the intelligence subtests Vocabulary and Coding, with lower scores being related to more severe ADI-total scores. Autism severity was also related to gender, with males having more severe autism symptomatology (Table 5). Regression analysis, entering Vocabulary and Coding as predictors and ADI-total as dependent variable, resulted in a model with R^2 =.075. Adding gender to the equation enlarged R^2 to .093. The negative coefficient of gender indicates that severity scores were higher for males (Table 6). Similarly, ADI-reciprocal social interaction was negatively associated with Vocabulary and Coding and multiple regression resulted in a model with R^2 =.086. Following the inclusion of gender increased R^2 to .106 (Table 6). Coding was negatively correlated with two ADI domains of impairment: reciprocal social interaction and stereotyped and repetitive behavior, with more severe impairments on the ADI domains related to weaker performances on Coding. Severity of repetitive behavior was negatively related to Information and Coding as well as to gender (Table 5), with more severe repetitive behavior related to poorer scores on Information and Coding. Again, males showed more severe symptoms of repetitive behavior. Including the subtest scores in the regression analyses predicting severity of repetitive behavior resulted in a model with R^2 =.062. Adding gender to the model increased R^2 to .081 (Table 6).

Severity of ADHD symptomatology in relation to intelligence

On factor level, no correlations were found between severity of ADHD symptomatology and intelligence (Table 4). On subtest level, Block Design was negatively correlated with total severity of ADHD symptoms (ADHD-total), and with the ADHD domains hyperactivity and impulsivity, with weaker performances on Block Design in children with more severe ADHD symptoms (Table 5). Severity of inattention problems was related to gender but not with any of the intelligence subtests (Table 5). Males had relatively more inattention problems than females. More severe hyperactivity symptoms were related to weaker performances on Arithmetic and Block Design. Regression analysis resulted in a model with R^2= .100 (Table 6). Moderation and mediation analyses were performed demonstrating that gender had no moderating or mediating role between subtests and severity of ASD or ADHD symptomatology.

Table 4. ***Correlation matrix with Pearson correlations of sex and age with ASD and ADHD severity and partial correlations of Full Scale-, Verbal-, Performance- and factor intelligence scores, controlling for sex with ASD and ADHD severity.***

	FSIQ	**VIQ**	**PIQ**	**VC**	**PO**	**PS**	**Sex**
Mean (SD)	66.0(12.3)	69.4(13.5)	67.7(12.5)	71.0(14.3)	69.3(12.7)	75.4(14.3)	-
ADI total	-.128	-.183*	-.098	-.185*	-.016	-.108	-.215*
Social interact	-.120	-.176*	-.093	-.177*	-.006	-.150	-.231*
Communication	-.093	-.124	-.094	-.120	-.033	-.037	-.153
Repetitive behaviour	-.089	-.139	-.008	-.142	.048	-.087	-.204*
ADHD total	-.066	-.003	-.091	.036	-.105	-.025	-.169
Inattention	-.014	-.033	-.053	.069	-.053	-.005	-.223*
Hyperactivity	-.102	-.026	-.098	-.007	-.139	-.036	-.127
Impulsivity	-.110	-.046	-.116	-.027	-.121	-.047	-.061

*Correlation is significant at the 0.05 level (1-tailed).

Table 5. ***Partial correlation matrix of Wechsler subtests, controlling for sex with ASD and ADHD domains***

	Information	Similarities	Arithmetic	Vocabulary	Comprehension	Digit Span	Picture Completion	Coding	Picture Arrangement	Block Design	Object Assembly	Symbol Search
Mean (SD)	5.0 (3.0)	5.4 (3.1)	4.1 (2.5)	4.0 (2.7)	4.9 (3.2)	6.8 (3.0)	5.1 (2.9)	5.5 (2.9)	4.9 (2.8)	4.9 (2.6)	5.4 (3.0)	5.4 (3.3)
ADI total	-.110	-.090	-.134	-.196*	-.090	-.063	-.103	-.172*	.077	-.018	-.030	-.014
Social interaction	-.078	-.081	-.126	-.204*	-.073	-.074	-.074	-.189*	.073	-.001	-.029	-.007
Communication	-.055	-.054	-.092	-.111	-.052	-.012	-.146	-.112	.054	-.014	-.046	-.051
Repetitive behaviour	-.177*	-.058	-.138	-.132	-.097	-.052	.085	-.173*	.104	-.079	-.013	-.028
ADHD total	.058	-.036	-.146	-.010	.115	-.086	-.048	-.035	-.041	-.228**	-.093	-.040
Inattention	.102	-.024	-.071	.051	.154	-.078	-.031	-.002	-.001	-.133	-.048	-.011
Hyperactivity	-.000	-.108	-.190*	-.011	.039	-.065	-.042	-.044	-.037	-.301**	-.155	-.074
Impulsivity	-.033	.014	-.181	-.081	.019	-.067	-.064	-.085	-.123	-.218*	-.065	-.043

* Correlation is significant at the 0.05 level (1-tailed) ** Correlation is significant at the 0.01 level (1-tailed).

Table 6. ***Regression analyses to predict ASD severity (Total severity, Social Interaction, Repetitive Behaviour) and ADHD severity (Hyperactivity) in subjects with 22q11DS.***

Total severity						
		F(df)	R^2	β	*p*	
	Step 1	3.532(2,87)	.075		.034	
	(constant)				.000	
	Vocabulary			-.157	.178	
	Coding			-.165	.158	
	Step 2	2.954(3,86)	.093		.037	
	(constant)				.000	
	Vocabulary			-.151	.193	
	Coding			-.115	.343	
	Sex			-.145	.191	
Social Interaction						
		F(df)	R^2	β	*p*	
	Step 1	4.071(2,87)	.086		.020	
	(constant)				.000	
	Vocabulary			-.157	.174	
	Coding			-.185	.110	
	Step 2	3.410(3,86)	.106		.021	
	(constant)				.000	
	Vocabulary			-.151	.189	
	Coding			-.133	.271	
	Sex			-.154	.162	
Repetitive Behaviour						
		F(df)	R^2	β	*p*	
	Step 1	2.869(2,87)	.062		.062	
	(constant)				.000	
	Information			-.098	.405	
	Coding			-.187	.115	
	Step 2	2.518(3,86)	.081		.063	
	(constant)				.000	
	Information			-.111	.346	
	Coding			-.128	.308	
	Sex			-.148	.187	
Hyperactivity						
		F(df)	R^2	β	*p*	
	Step 1	4.822(2,87)	.100		.010	
	(constant)				.000	
	Arithmetic			-.039	.755	
	Block Design			-.292	.021	

Discussion

This study evaluated domains of intellectual functioning of 102 individuals with 22q11DS, investigating differences between subgroups with and without symptoms of attention deficit hyperactivity disorder (ADHD) and autism spectrum disorder (ASD). Further, the relation between intelligence on factor and subtest levels of intelligence with severity of ASD and ADHD symptomatology was explored. Outcomes revealed no significant differences in intelligence profiles between participants with and without ASD or a dual diagnosis ASD and ADHD. For the total group a higher mean score on Processing Speed and a lower score on Perceptual organization was found, relative to the mean factor score. On subtest level, a significantly higher score was found on Digit Span together with lower scores on Arithmetic and Vocabulary, relative to the mean subtest score. Notable, these profiles did not differ between participants with and without ASD and/or ADHD, which enabled us to examine the relation between intelligence profiles and severity of ASD and ADHD symptoms in the total sample. Lower scores on Vocabulary and Coding were related to more severe ASD symptoms, while lower scores on Block Design and Arithmetic were related to more severe ADHD symptomatology.
In participants with 22q11DS intelligence did not discriminate between individuals with and without ASD and/or ADHD. However, the intelligence profile differed from the intelligence profiles reported in idiopathic ASD and ADHD groups. In those groups, lower scores were reported for Coding relative to Symbol Search in ADHD as well as ASD (Calhoun & Mayes 2005; Oliveras-Rentas *et al*. 2012) while this association was not found in our group with 22q11DS with or without developmental disorders. Also the typical finding of low Comprehension scores relative to Block Design scores in idiopathic ASD populations (Calhoun & Mayes 2005; Oliveras-Rentas *et al*. 2012) was not found in the 22q11DS sample. Possibly, these differences can be explained by the fact that in our study a genetically defined subgroup was selected from an otherwise idiopathic and heterogeneous population of individuals with ASD or ADHD. Another explanation for this difference could be that most studies in idiopathic ASD or ADHD populations investigated relative high functioning individuals, while the current 22q11DS sample (mean FSIQ of 68) was functioning on a below average cognitive level.
In contrast to the study of Niklasson and Gillberg (2010), no differences between participants with or without ASD or ASD and ADHD were found. They also investigated intelligence on subtest level in individuals with 22q11DS with or without ASD and ADHD, but compared subtest scores within factors against each other instead of comparing all subtests with the mean subtest score (our study). Therefore, both studies are not fully comparable. Our finding of relatively higher scores on Digit Span and lower scores on Arithmetic in children with 22q11DS, consistent with findings of Niklasson and Gillberg, suggests relatively stronger quality of functions that are used for Digit Span such as quality of short term attention and memory in individuals with 22q11DS. On the other hand functions involved in Arithmetic such as concentration during a longer period and long term memory seem to be weaker (Sattler 2001). The lower scores on Vocabulary suggest that participants with 22q11DS are less able to understand or express the meaning

of individual words relative to their overall intellectual capacities (Sattler 2001). However, this finding was not supported by the study of Niklasson and Gillberg who found superior scores on Vocabulary in their ASD and No ASD/ADHD groups. This might be explained by the different age ranges (7-35 years - Niklasson & Gillberg vs. 9-18 years in our study). Decreases in Vocabulary and Arithmetic were reported in a longitudinal study between age 7.5 and 9.5 years by Duijff *et al*. (2012), who argued that the overall cognitive decline may be explained by a progressive delay in verbal comprehension and expression. The current study shows a comparable weakness in Vocabulary and Arithmetic. Underlying mechanisms of these poor performances could be poor verbal comprehension or difficulties in verbalization. However, the performance on subtests of intelligence requires multiple cognitive functions and could also be influenced by factors like anxiety or poor concentration (Sattler 2001). It is therefore necessary to investigate the underlying mechanisms of cognitive functions involved in the subtests such as executive function skills.

A second aim of this study was to expand existing knowledge by investigating the relation between performances on subtest levels of intelligence and severity of ASD and ADHD symptomatology. Regression analysis indicated a negative association between quality of reciprocal social interaction in individuals with 22q11DS and performances on Vocabulary and Coding, what might suggest that poorer perceptual-motor integration of visual information processing and more difficulties with verbal comprehension or expression are possible underlying mechanisms of more severe problems with reciprocal social interaction. It is difficult to determine which cognitive abilities are exactly involved because performance on subtests depends on multiple functions (e.g. Coding performances may also result from poor pencil control, poor motivation or impulsivity). However, the consistent finding of relations between specific subtests and severity measures which resulted in regression models explaining up to 10% of the variance are an important contribution in exploring the relation between cognitive problems and the vulnerability to developmental disorders in 22q11DS. Poorer performances on the subtests Information and Coding were associated with increased severity of repetitive and stereotyped behavior. This implies that the presence and severity of these behaviors is associated with the quality of general factual knowledge and quality of long-term memory in children with 22q11DS as well as with their short-term visual memory, accuracy and attention capacities. Performances on the subtest Block Design were negatively related to total ADHD severity as well as to severity of hyperactivity and impulsivity. Weaker visuospatial information processing in individuals with 22q11DS therefore seems to be related to ADHD symptomatology. Hyperactivity was also influenced by processes that subserve performance on Arithmetic. These processes are poor numerical reasoning, concentration, attention, short and long term memory.

Gender was related to total autism severity, difficulties in reciprocal social interaction and repetitive and stereotyped behavior as well as to inattention. Higher severity scores were found on these domains for males. They also had lower scores on the intelligence measures. No moderation or mediation of gender was found. The relations between cognitive functioning and severity scores of males and females are both in the same direction. Our data suggest this relation is less strong for females,

although this effect was not significant. These differences between males and females contrasts with the results of studies that did not find a relation between gender and intelligence in 22q11DS (De Smedt *et al*. 2007; Moss *et al.* 1999; Niklasson *et al*. 2005), but are in line with findings of others (Antshel *et al*. 2005a; Duijff *et al*. 2012; Niklasson & Gillberg 2010). No explanation for these gender differences is suggested yet and it therefore remains important to look at gender when assessing individuals with 22q11DS, especially because the syndrome is not gender-specific, meaning that females and males are equally represented in the 22q11DS population.

Strengths and limitations

The investigation of ASD and ADHD symptoms in 22q11DS and the relation to intellectual functioning is a valuable contribution to the understanding of developmental disorders in 22q11DS. Given the high rate of co-morbid occurrence of ASD and ADHD symptoms in this syndrome, we investigated the co-occurrence of both disorders and their relation to cognitive abilities in children with 22q11DS. This study supports the approach of the DSM-5 which provides the opportunity to specify other associated disorders, separately classifying autism or ADHD. It also proves the usefulness of defining severity of diagnostic symptoms of both disorders, as is required by the DSM-5 (American Psychiatric Association 2013). A limitation of this study could be the absence of a control group. However, it is difficult to determine whether such a control group should be matched on age, intelligence, developmental age or on other characteristics that makes this group unique by its syndrome specific features. Providing insights in functioning on different aspects within the syndrome seems more relevant than comparing these children to control populations.

Conclusion and implications

From this study it can be concluded that investigating intelligence in relation to severity of developmental disorders in 22q11DS can contribute to our understanding of this complex disorder. Consistent with previous studies intellectual functioning does not discriminate between 22q11DS participants with and without ASD and ADHD (Vorstman *et al*. 2006; Gothelf *et al.* 2007; Green *et al.* 2009; Hooper *et al.* 2013). However, the current study demonstrates that on subtest level, intelligence is related to severity of the symptomatology of these disorders in 22q11DS. Poorer performance on different aspects of cognitive functioning is related to higher severity of the different symptom domains of these developmental disorders. From these findings it is recommended to focus on multiple and more detailed levels of cognitive functioning in evaluating the developmental impact of 22q11DS. Intelligence is a global measure of cognitive functioning and our findings on subtest level are promising in that specific aspects of cognitive functioning seems to be related to the severity of autism and ADHD symptomatology. It is likely that both ASD and ADHD symptoms are present in individuals with 22q11DS at varying levels of severity, and sometimes without an explicit diagnosis (Baker & Vorstman 2012). Hence, focusing on the severity of this symptomatology seems relevant and

can provide new and valuable insights into the relation between cognitive functioning and this psychopathology. Expanding the investigation of these relations to underlying mechanisms of cognitive abilities such as executive functioning is recommended. In the current study intelligence performances on subtest level explained only up to 10% of the variance in ASD and ADHD symptomatology. This suggests that other factors or mechanisms may also be contributing to the severity of the symptomatology. The presented evidence for the relations between cognitive function profiles and severity of symptomatology may have clinical implications in that it may help to adjust treatment strategies and demands to the needs of the individual. Knowledge of the cognitive strengths and weaknesses of an individual may provide a starting point for the development of interventions that may possibly be customized to suit individual needs and enables to formulate realistic expectations of the effect interventions might have. In addition, this knowledge may help monitoring cognitive development of individuals during different stages of life and finally lead to a better adjustment of demands to the capacities of the individual.

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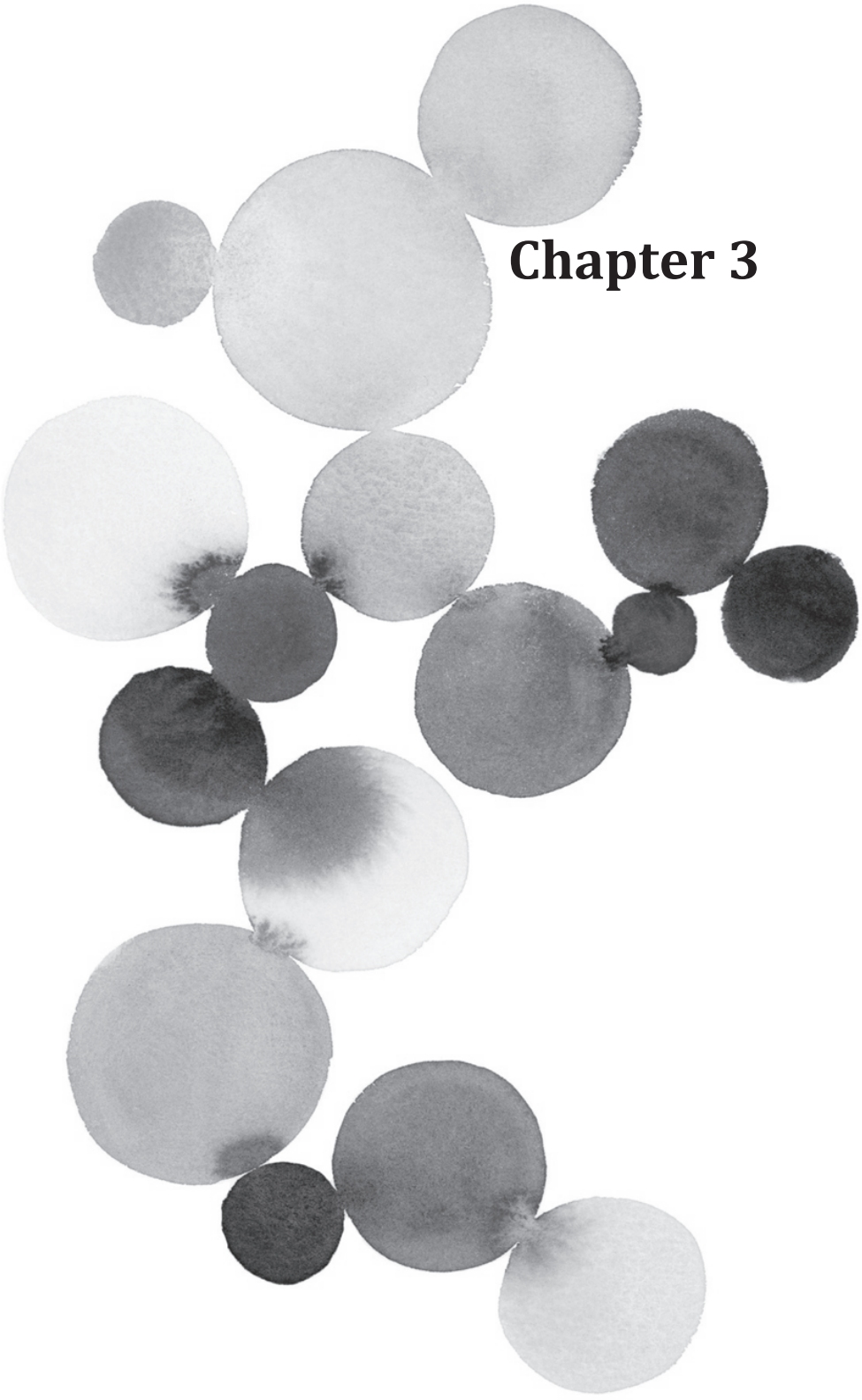

Chapter 3

Executive functioning and its relation to autism and ADHD symptomatology in 22q11.2 Deletion Syndrome

Hidding, E., de Sonneville, L. M. J., van Engeland, H., Vorstman, J. A. S., Sijmens-Morcus, M. E. J., & Swaab, H. Executive functioning and its relation to autism and ADHD symptomatology in 22q11.2 Deletion Syndrome. *Under review.*

Abstract

Children with 22q11.2 deletion syndrome (22q11DS; velo-cardio-facial-syndrome) are at risk for the developmental disorders attention-deficit-hyperactivity disorder (ADHD) and autism spectrum disorder (ASD). In the present study the relation between executive functioning (EF) and severity of ADHD and ASD symptoms was examined, since EF is known to be important in relation to emotional and behavioral problems.
58 children (38 females) with a mean age of 13.5 (SD 2.6) years participated. Standardized assessment was used to evaluate the presence of ASD and ADHD symptomatology. Major aspects of EF, including cognitive flexibility, inhibition, sustained attention, distractibility, working memory, reaction speed, perseveration, and planning were evaluated.
The profile of EF in 22q11DS was characterized by weaker performance, compared to the norm, on all subdomains of EF, except for perseveration. Poor cognitive flexibility and inhibition, and high distractibility were found to be related to more severe ASD symptoms, while poor quality of sustained attention, and high distractibility were related to more severe ADHD symptoms.
Children with 22q11DS experience impairments in EF and the degree of impairment on specific EF subdomains is related to severity of ASD or ADHD symptomatology. These results may help in defining the mediating role of neurocognitive dysfunctions in the development of social and behavioral problems in 22q11DS.

Background

Children with the congenital genetic disorder 22q11.2 deletion syndrome (22q11DS) are at risk for developmental disorders such as attention-deficit-hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) (Antshel *et al.* 2006; Antshel *et al.* 2007; Niklasson *et al.* 2009). Already in childhood and adolescence there is a substantially higher prevalence, compared to typical controls, of different behavioral and emotional problems, such as problems in attention regulation, impulsivity, communication and social interaction, that are part of ADHD and ASD (Antshel *et al.* 2007; Fine *et al.* 2005; Vorstman *et al.* 2006). Because it is widely known that genetic factors are involved in those developmental disorders, investigating a genetic syndrome that is associated with symptoms of these disorders is an unique opportunity to improve our knowledge about the neural basis of these disorders (Rutter 1997; Scourfield 1999). Especially, investigating neuropsychological dysfunctions as possible underlying mechanisms of the behavioral and emotional problems of those disorders in 22q11DS may provide insight in the etiology of ASD and ADHD. Deficits in these executive functions that regulate behavior and thought (Blakemore and Choudhury 2006; Anderson 2001), are found to underlie behavior and adaptation problems observed in ADHD (Barkley 1997; Sonuga-Barke 2003) and ASD (Ozonoff *et al.*1991; Hill 2004; Gargaro *et al.* 2011). EF could therefore be important in determining vulnerability to ASD and ADHD symptomatology in individuals with 22q11DS. Insight in this relation may provide opportunities to develop interventions that improve cognitive functioning in children with 22q11DS and may lead to a better developmental outcome. Recently, one preliminary study reported gains in cognition after a cognitive remediation program in adolescents with 22q11DS (Harrell *et al.* 2013).

Across studies in 22q11DS, a broad range of EF has been investigated with different aspects studied in different samples. Dysfunctions have been found in processing speed, cognitive flexibility, mental set-shifting, sustained and selective attention, working memory, inhibition, planning and problem solving (Ousley *et al.* 2007; Woodin *et al.* 2001; Rockers *et al.* 2009; Campbell *et al.* 2010; Lewandowski *et al.* 2007; Shashi *et al.* 2010; Antshel *et al.* 2008; Niklasson *et al.* 2005; Furniss *et al.* 2011; Stoddard *et al.* 2011; Lajiness-O'Neill *et al.* 2006; Sobin *et al.* 2005; Gur *et al.* 2014). The heterogeneity in methods precludes to determine a clear EF profile and may have contributed to the lack of consistent patterns in findings so far. For example, in some studies response inhibition has been reported to be impaired in 22q11DS (Sobin *et al.* 2005; Antshel *et al.* 2008; Campbell *et al.* 2010), whereas in other studies such impairment was not found (Lajiness-O'Neill *et al.* 2006; Gothelf *et al.* 2007).

Importantly, a relation between executive dysfunctions and developmental disorders in 22q11DS has not convincingly been demonstrated yet. Therefore, a study investigating multiple aspects of EF in individuals with 22q11DS is necessary to unravel the relation between executive dysfunctions and behavioral outcomes in 22q11DS. Especially because only a few studies have focused on EF in relation to ASD and ADHD symptomatology. Results thus far suggest that EF deficits are different for individuals with and without psychopathology. For example, in a study that did not differentiate between individuals with and without psychopathology planning ability was found to be impaired in 22q11DS (Henry *et al.* 2002). Indeed, in a subsequent study, planning ability was found to be impaired *only* in those children who also had ASD/ADHD symptoms, while children without these symptoms had average planning abilities (Niklasson and Gillberg 2010). This suggests a relation between EF and ASD and ADHD symptomology in 22q11DS and underlines the importance of examining this issue further including a wide range of EF.

Differences in EF within the 22q11DS population may also depend on age since EF develops with age as a result of the ongoing development of the brain during childhood and adolescence (Anderson 2001; Best and Miller 2010). It can be argued that differences in EF could also explain differences in developmental trajectories within this population. Investigating executive aspects of attention in relation to age, Stoddard et al. (2011) found more pronounced impairments in younger children with 22q11DS (age range 7-14 years). In a longitudinal study it was shown that some but not all cognitive performances of individuals with 22q11DS declined with age: learning and memory skills did, but perseveration and planning improved (Antshel *et al.* 2010).

In conclusion, studying the relation between EF and behavior in subjects with 22q11DS may help to clarify the relation between a genetic factor (22q11DS) and the development of social and behavioral problems through the mediating role of neurocognitive dysfunctions. Importantly, knowledge about the specificity of impairments in EF and its relation to vulnerability to ASD and ADHD symptoms provides an opportunity to develop cognitive interventions for these children. The aim of our study was to extend previous findings by the evaluation of a wide range of EF, focusing on the relation between EF and severity of ADHD and ASD symptoms. In line with previous results we anticipated that EF is impaired in individuals with 22q11DS. Based on the lack of consistent patterns in findings so far, we expected that some but not all of the EF included in the assessment are impaired. We hypothesized that poorer EF is associated with increased severity of ASD and ADHD symptoms. More specifically, based on research thus far, we expected impairments in working memory and inhibition to be related to more severe ADHD symptoms and impairments in planning, inhibition and flexibility to be related to more severe ASD symptoms. We

also explored the relation between dysfunctions in EF and age because of inconsistencies in findings thus far. Since other studies have not found sex differences in relation to EF in 22q11DS, we did not expect to find an effect of sex (Woodin *et al.* 2001; Niklasson and Gillberg 2010).

Methods

Sample

In this study 58 children (38 females, Age: *M*=13.48; *SD*=2.6; *min* = 9; *max* =18.5, FSIQ: *M*=65.2; *SD*=13.3) with 22q11DS, as confirmed with a fluorescence in situ hybridizations, participated. The study was part of a nationwide study. Recruitment took place at the Department of Psychiatry, Brain Center Rudolph Magnus of the University Medical Centre Utrecht (UMCU) as well as through a request that was posted on the website and in the newsletter of the 22q11DS parents' network in the Netherlands. Parents and participants were informed by phone about the aims of the study and received a complete description of the study in writing before they decided on participation. Informed consent was obtained from participants and parents or caretakers. The assessment protocol was approved by the Dutch Central Committee on Research Involving Human Subjects. Assessments took place at the outpatient center of the UMCU and were carried out by an experienced child neuropsychologist and child psychiatrist. At the time of assessments 3 children were treated with atypical antipsychotics and 1 with stimulant medication. Other medication used by participants were anti epileptics (n=1), Beta blocker (n= 1) and thyroid medication (n= 2).

Measures

Psychiatric classifications were made according to DSM-IV criteria, resulting from a multidisciplinary consensus meeting headed by an experienced child psychiatrist, on the basis of clinically structured and semi-structured interviews (with both the child and the caregivers), observation of the child questionnaires and intelligence assessment.

The assessment protocol included *the Autism Diagnostic Interview-Revised* (ADI-R) (Rutter *et al*. 2003), scored by certified interviewers. The ADI-R provides algorithmic scores for the three domains in which children with ASD experience difficulties (reciprocal social interaction, communication impairment, repetitive and stereotyped behaviors), which were used to quantify autistic symptoms (Rutter *et al*. 2003).

Classifications of autism and pervasive developmental disorder not otherwise specified are both referred to as ASD.
In addition the *Schedule for Affective Disorders and Schizophrenia for School-Age-Children-Present and Lifetime* Version (K-SADS-PL) (Kaufman *et al*. 1997) was used to quantify psychotic symptoms.
Furthermore, information from the caregivers and the teachers was obtained using the Child Behavior Checklist, the Teacher Rating Form (Achenbach 1991; Achenbach & Rescorla 2001) and Conners' Rating Scales-Revised (CRS-R; Conners 1997).
Intellectual functioning was assessed, using a current version of the Wechsler Intelligence Scale (Wechsler 2002; Wechsler 2005b; Wechsler 1974) for children and the Wechsler Adult Intelligence Scale-III (Wechsler 2005a) for adolescents older than 16 years.
An overview of the formal psychiatric classifications of the sample is provided in Table 1, reflecting the multidisciplinary clinical consensus based on all available patient information.

Severity of ASD and ADHD symptomatology

In some cases, the formal diagnoses deviate from the classifications that would be obtained if only the outcomes of the questionnaires were used. The DSM-IV guidelines do not allow diagnosing ADHD and ASD in the same individual (American Psychiatric Association 2000). As a result, in most cases with prominent ASD symptomatology and ADHD symptoms, only a formal diagnosis of the former was made. In two individuals ADHD symptoms were prominent justifying a formal (comorbid) diagnosis of ADHD (Table 1).
Because of the high prevalence of both ASD and ADHD symptoms in 22q11DS the possible co-occurrence of symptoms of both neurodevelopmental disorders was also investigated. To this end, we used the three ADHD domains (inattention, hyperactivity, impulsivity) as rated with a semi-structured interview based on the criteria of DSM-IV as a measure of severity of ADHD symptoms. The interview consisted of items comparable to those of the CRS-R (Conners 1997) and the Dutch version of the ADHD DSM-IV rating scale (Kooij *et al.* 2008). Likewise, the '4.0 to 5.0/ever' algorithmic scores of three domains of the ADI-R were used as a measure of autism symptoms (Rutter *et al*. 2003; McDuffie *et al.* 2010). Table 2 provides the means and distribution of the ASD and ADHD severity scores.

Table 1 ***Psychiatric classifications according to DSM-IV criteria with primary diagnoses and comorbid diagnoses.***

Diagnostic classification (primary)		*Comorbid diagnoses***				
	N	ASD	ADHD	Dep.dis	ODD*	Psych.dis
Autism spectrum disorder (ASD)	31		2	4	1	5
Attention Deficit Hyperactivity Disorder	1					
Anxiety Disorder	1					
Conversion Disorder	1					
Depressive disorder (Dep.dis)	2					
Psychotic disorder (Psych.dis)	2					
Without psychiatric classification	20					
Total	58	0	2	4	1	5

* Oppositional defiant disorder ** Represent comorbid diagnoses within the total N of 58

Table 2 ***Autism and ADHD severity scores.***

	N	M	SD	Range
ADHD-total	57	11.47	8.34	0-30
Inattention	57	7.75	6.06	0-23
Hyperactivity	57	1.84	2.32	0-8
Impulsivity	57	1.88	2.13	0-9
ADI-total	58	24.17	13.35	0-49
Reciprocal social interaction	58	10.76	6.92	0-26
Communication impairment	58	7.47	5.05	0-19
Repetitive and stereotyped behaviors	58	2.12	2.06	0-8

Executive Functioning

The Amsterdam Neuropsychological Tasks (ANT) program (De Sonneville 1999; De Sonneville 2005) was used to evaluate major components of executive functioning (EF), i.e., alertness, sustained attention, working memory, distraction, inhibition, and cognitive flexibility. The ANT has been proven to be a well-validated and sensitive instrument to evaluate attentional processes and EF in psychiatric disorders such as ADHD (Slaats-Willemse *et al.* 2007) and ASD (Van Rijn *et al.* 2013). Test–retest reliability, construct-, criterion-, and discriminant validity of the computerized ANT are satisfactory and have extensively been described and illustrated elsewhere (Gunther *et al.* 2005; Huijbregts *et al.* 2002; Rowbotham *et al.* 2009; De Sonneville 2014). To obtain a measure of perseveration and planning skills, the Wisconsin Card

Sorting Test (WCST) (Heaton *et al.* 1993) and the Rey-Osterrieth Complex Figure (RCFT) (Rey 1964) were used.
Alertness was evaluated using the Baseline Speed task (BS) (Van Rijn *et al.* 2013; Gunther *et al.* 2011), which is a simple reaction time task. A fixation cross presented on a screen changes unexpectedly into a square, the imperative signal. The child is instructed to press a mouse key as fast as possible when the square appears. Reaction speed is operationalized as the mean reaction time (RT) to signals. Fluctuation in reaction speed is operationalized as the within subject standard deviation (SD) of RT across the 32 trials.
Sustained attention was assessed using the SA-dots task (SAD) (Van Rijn *et al.* 2013). This task measures the ability to maintain performance at a certain level during a longer period of time. During this task 600 random patterns of 3, 4 or 5 dots are successively presented in 50 series of 12 trials. Children are required to respond to the 4-dots pattern (target) by pressing the mouse button with their preferred hand ('yes'-response) and to the 3- or 5-dots patterns (nontargets) by pressing the mouse with their non-preferred hand ('no'-response). The ratio targets/nontargets is 1/2 which invokes a response bias to press the 'no-key'. Failure to inhibit this 'prepotent response' is expected to result in the production of relatively more misses than false alarms (De Sonneville *et al.* 1994). Task duration is approximately 15-20 minutes. Main outcome measures are mean series completion time (tempo), within-subject SD of tempo across 50 series (fluctuation in tempo) as measures of sustained attention, impulsivity (misses) and poor stimulus evaluation (false alarms).
Inhibition of prepotent responses and Cognitive flexibility were measured with the Shifting Attentional Set Visual task (SSV) (Huijbregts *et al.* 2010). During trials a colored square moves across a horizontal bar in the center of a screen, randomly to the right or left. The task consists of three parts. In part 1 (fixed compatible condition) the child is asked to follow the movement of a green block by pressing the left button upon a left move and the right button upon a right move. In part 2 of the task (fixed incompatible condition), using a red block, the child is asked to do the opposite, i.e. 'mirror' the movement of the block, by pressing the left button upon a right move and vice versa, requiring the inhibition of prepotent responses. Inhibition is operationalized as the contrast in performance (speed/accuracy) between part 1 and part 2. In part 3 (random condition), the block changes color randomly asking the child to follow or 'mirror' the movement, depending on the color of the block. In this part the child needs to shift response sets, i.e. to readily switch between execution of a prepotent response and inhibition of a prepotent response (in favor of the requested response), which switch requires cognitive flexibility. Cognitive

flexibility is operationalized as the contrast in performance between part 1 and part 3.
Working Memory and Distraction were measured using the Memory Search Letters task (MSL) (De Sonneville *et al.* 2002). This letter detection task consists of three parts increasing the memory load from one item in part 1 (k), to two items in part 2 (k+r), and three items (k+r+s) in part 3. The display set of four letters that contains the complete target set requires a 'yes'-response, incomplete target sets requires a 'no'-response. Target letters in nontarget trials act as distractors. Memory search rate is operationalized as the contrast in speed/accuracy of responses to target signals in part 1 (low load) and part 3 (high load). Distraction is operationalized as the contrast in speed/accuracy of responses to nontarget signals in part 3 between signals with 0 distractors (low distraction) and two distractors (high distraction).
Planning was operationalized as the accuracy copy score of the Rey Complex Figure test (RCFT) (Rey 1964). Children are instructed to copy an abstract figure as accurately as possible. Accuracy of the drawing was scored according to the Taylor scoring criteria (Straus *et al.* 2006).
Perseveration was measured using the Wisconsin Card Sorting Task (WCST) (Heaton *et al.* 1993). Perseveration was measured by contrasting the number of perseverative errors and non-perseverative errors. Perseverative errors are made when the child continues sorting the cards based on a previously succesful principle or initial erroneous guess in the first serie (Lezak *et al.* 2012, Barneveld *et al.* 2013). Thus, in this task perseveration is operationalized as the inability to discontinue the use of a certain strategy in favor of another one despite feedback prompting to do so, with both strategies not being associated with prepotency (as is the case in task SSV).

Statistical analyses

Main outcome parameters of the ANT-tasks, the RCFT and the WCST were transformed to z-scores (De Sonneville 2005; De Sonneville 2014; Strauss *et al.* 2006). For the ANT the z-scores that were entered in the analyses are the results of computations, based on nonlinear regression functions that describe the relation between test age and task performance. These functions are fully implemented in the ANT program, based on norm samples varying in size between 3,100 to 6,700 subjects, depending on the task, and are therefore considered to be reliable estimates of performance level.
Results on each ANT-task were examined for extreme values. As extreme values are a clinical reality in this population, z-scores ≥ 6 were set to 6 to keep these subjects in

the analyses (Table 3). Not all subjects completed the entire assessment battery, therefore degrees of freedom will vary between analyses. Subjects with substantial missing data were excluded from analyses (n=5), resulting in a final sample of n=58. In addition, missing values in the final sample are the consequence of an inability of the subject to complete difficult task parts, or skipping parts because of running out of time.

Comparison to the norm

To decide whether mean performance of the subjects with 22q11DS differed from the norm, i.e. differed from zero for z-scores, the intercept test of the (M)ANOVAs was used. Alpha was set to 0.01. Multivariate group effects were analyzed using Pillai's trace. Effect sizes were calculated using partial eta squared with $\eta p^2 \sim 0.03$ representing a weak effect, $\eta_p^2 \sim 0.06$ representing a moderate effect and $\eta_p^2 \geq 0.14$ significantly a large effect (Cohen 1992). ANOVA's were used for all *post hoc* analyses of group effects. Prior to analysis, assumptions for the analyses were examined and confirmed to be satisfactory.

Alertness: mean RT and fluctuation of RT during Baseline Speed were entered as dependent variables in a MANOVA.

Sustained Attention: Tempo and fluctuation in tempo were entered as dependent variables in MANOVA of speed. Number of misses and number of false alarms were entered as dependent variables in MANOVA of accuracy.

The results of the remaining ANT tasks were analyzed using Repeated Measures ANOVAs. Separate runs were made with RT and accuracy (errors) as dependent variables. The within-subject (WS) factors were, respectively:

Cognitive flexibility: contrast between performance in Part 1 (compatible responses) and Part 3 (compatible responses)

Inhibition: contrast between performance in Part 1 (compatible responses) and Part 2 (incompatible responses).

Memory load: contrast between performance on target signals in Part 1, 2 and 3

Distraction: contrast of performance on nontarget signals in Part 3 with 0, 1 and 2 distractors.

A significant WS effect reflects that task conditions result in different levels of performance. As z-scores are used, this implies that differences in performance between patients and the norm depend on task condition/level (interaction).

Planning: Planning score was entered as dependent variable in an ANOVA.

Perseveration: The percentage perseverative errors and non-perseverative were entered as levels of the WS factor *Perseveration* in a repeated measures ANOVA.

Severity of ASD and ADHD symptomatology

Pearson correlations were calculated in order to assess the relation between severity of ASD and ADHD symptoms and EF (small effect size: *r*=0.1-0.23; medium: *r*=0.24-0.36; large: *r*≥ .37, Cohen 1992). In case of significant correlations, regression analysis was performed to test the relation between EF and severity of ASD or ADHD symptoms, respectively. Prior to these analyses we examined whether age, full scale IQ and sex were correlated with *both* EF and symptom severity.

Table 3 ***Distribution of scores on EF across the standard deviations (SD) in %.***

		≤1SD	≥2SD	≥6SD
Reaction Speed	RT	52.6	28.1	5.2
	Fluc	56.1	31.6	7.0
Sustained Attention	Tempo	35.1	47.4	7.0
	Fluc	29.8	43.9	5.3
	Miss	64.9	17.5	1.8
	FA	78.9	10.5	1.8
Attentional Flexibility	RTC1	69.2	9.6	0.0
	RTC3	75.6	8.9	2.2
	AccC1	69.2	15.4	1.9
	AccC3	22.9	66.7	27.1
Inhibition	RTC1	69.2	9.6	0.0
	RTI2	75.5	16.3	2.0
	AccC1	69.2	15.4	1.9
	AccI2	28.8	53.8	25.0
Working Memory	RT1	71.4	10.7	1.8
	RT3	71.9	10.5	1.8
	Acc1	66.7	12.3	1.8
	Acc3	78.9	8.8	5.3
Distraction	RT0	73.2	14.3	1.8
	RT2	75.0	16.1	1.8
	Acc0	87.7	5.3	3.5
	Acc2	68.4	15.8	5.3
Planning		28.1	38.6	10.3
Perseveration	Perr	87.0	12.1	0
	NPerr	83.3	3.4	0

Note: Scores for Speed (RT), Tempo, Fluctuation in speed or tempo (Fluc), Misses (Miss), False alarms (FA), Accuracy (Acc.), Perseverative errors(Perr) and NonPerseverative errors (NPerr). C1, C3 compatible condition part 1 and part 3 (SSV); I2 incompatible condition part 2 (SSV); 1,3 part 1 (low load condition) and 3 (high load condition)(MSL); 0,2 part 3 with 0 distractors (low distraction condition) or 2 distractors (high distraction condition)(MSL)

Results

Standardized means of total group performances on all executive functioning tasks are presented in Figure 1. Negative deviations from zero indicate more efficient EF, while positive deviations reflect worse performances. An overview of the distribution of scores across the standardized scores is presented in Table 3.

Alertness

Subjects with 22q11DS were slower [F(1,56)=28.421, p<.0001, η_p^2 =.337] and showed more fluctuation in reaction speed [F(1,56)=27.388, p<.0001, η_p^2 =.328] as compared to the norm (Fig.1).

Sustained Attention

Subjects with 22q11DS demonstrated a slower tempo [F(1,56)=61.761, p<.0001, η_p^2 =.524] and more fluctuation in tempo [F(1,56)=68.278, p<.0001, η_p^2 =.549] as compared to the norm (Fig. 1). They also made more misses than the norm [F(1,56)=6.989, p=.011, η_p^2 =.111], but not more false alarms (p=.170) (Fig. 1), suggesting a difficulty to keep the response bias (increasing during time-on-task) under control.

Cognitive Flexibility

Regarding speed, the WS factor Flexibility was significant [F(1,44)=7.082, p=.011, η_p^2 =.139], indicating that the 22q11DS sample did (slightly) better than the norm when flexibility was required (Fig.1). The average speed of the 22q11DS sample did not differ from the norm (p=.699). Regarding accuracy, the mean performance was less accurate compared to the norm [F(1,47)=84.984, p<.0001, η_p^2 =.644]. The effect of Flexibility was significant [F(1,47)=58.723, p<.0001, η_p^2 =.555], reflecting a steep increase in error rate compared to the norm when flexibility was required (Fig.1).

Inhibition

Regarding speed, the effect of Inhibition was not significant (p=.974) and mean performance of the 22q11DS sample was not significantly slower compared to the norm (p=.041). The 22q11DS sample made more errors compared to the norm

[F(1,51)=68.536, p<.0001, η_p^2=.573]. An effect of Inhibition was found, with a decrease in accuracy compared to the norm when inhibition demands were high [F(1,51)=38.733, p<.0001, η_p^2=.432].

Working Memory

On speed, subjects with 22q11DS performed slower as compared to the norm [F(1,55)=7.788, p=.007, η_p^2=.124] (Fig. 1). No effect of Memory load was found (p =.217), indicating that memory load did not discriminate between patients and the norm. Regarding accuracy, the effect of Memory load was significant [F(1,56)=7.080, p=.010, η_p^2=.112], reflecting a larger decrease in accuracy compared to the norm with memory load (Fig. 1). Mean accuracy of the 22q11DS was not significantly lower as compared to the norm (p=.028)

Distraction

The 22q11DS sample was on average slower as compared to the norm [F(1,54)=10.028, p=.003, η_p^2=.157]. No effect was found for Distraction (p=.397), indicating that the presence of distractors did not differentiate the 22q11DS sample from the norm on speed (Fig.1).

Mean accuracy across distraction conditions of the subjects with 22q11DS did not differ as compared to the norm (p=.946), but an effect of Distraction was found for the 22q11DS sample [F(1,56)=26.521, p=.0002, η_p^2=.321] reflecting that the unfavorable effect of distraction on accuracy was larger in the 22q11DS sample compared to the norm (Fig.1).

Planning

The 22q11DS sample performed poorer on planning as compared to the norm [F(1,56)=46.009, p<.0001, η_p^2=.451] (Fig.1).

Perseveration

Subjects with 22q11DS did not differ from the norm on perseveration (p=.043, Fig.1).

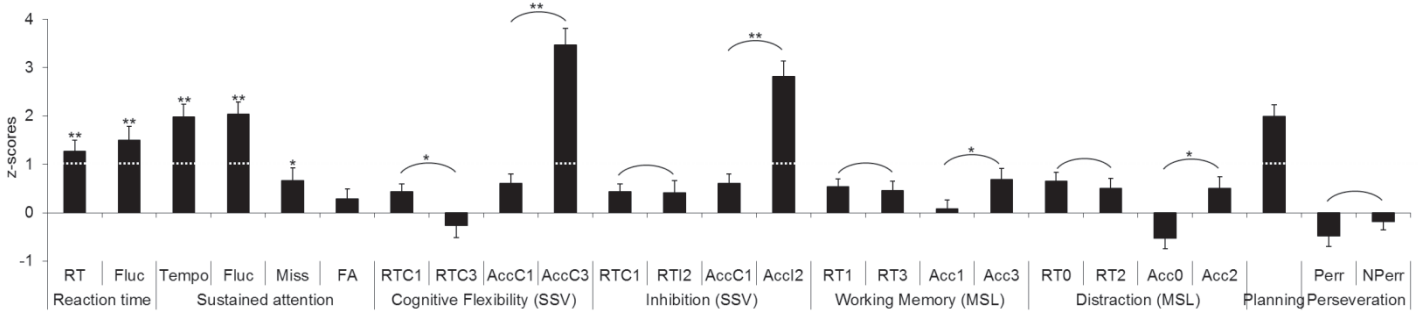

Figure 1 Mean z-scores of the total group showing level of performance on Executive Functioning

With scores for Speed (RT), Tempo, Fluctuation in speed or tempo (Fluc), Misses(Miss), False alarms (FA), Accuracy (Acc.), Perseverative errors(Perr) and NonPerseverative errors (NPerr). * Significant different from norm population at p <0.01, ** Significant at p<0.001.

Important contrasts between tasks conditions are indicated by the curved line elements. C1, C3 compatible condition part 1 and part 3 (SSV); I2 incompatible condition part 2 (SSV); 1,3 part 1 (low load condition) and 3 (high load condition)(MSL); 0,2 part 3 with 0 distractors (low distraction condition) or 2 distractors (high distraction condition)(MSL).

Age and full scale IQ in relation to executive functioning

A positive correlation was found between age and performances on fluctuation in reaction speed, tempo of sustained attention and planning ($p \leq .01$). This indicated that older children performed worse on these EF tasks. Reaction speed, sustained attention, working memory, and planning were correlated with full scale IQ, indicating that children with a lower full scale IQ performed worse on these EF tasks,which is not surprising since executive functions are needed to perform intelligence tests. Beside the reasons for not including IQ as a covariate as argued by Dennis et al. (2009), both age and full scale IQ were not correlated with severity of ASD or ADHD symptoms and were therefore not included in the regression models.

Severity of autism symptomatology in relation to executive functioning

A more severe ADI-total score was associated with decreases in speed when flexibility or inhibition was required (.05 level, Table 4), but regression analysis with these variables resulted in a non-significant model with R^2 =.069 (p=.257). Decreases in speed when flexibility or inhibition was required correlated in a similar way to Reciprocal social interaction (Table 4) with a non-significant regression model with R^2 =.087 (p=.177). A more severe Communication impairment was related to decreases in speed when inhibition was required and when distraction was present as well as to an increase in accuracy when flexibility was required (Table 4). Regression analysis with these variables resulted in a non-significant model with R^2 =.158 (p=.107). No relation between Repetitive and stereotyped behaviours with any of the EF measures was found.

Severity of ADHD symptomatology in relation to executive functioning

Higher scores on Hyperactivity and Impulsivity were significantly correlated to an increase in accuracy when memory load increased, a decrease in speed when distraction was present (Table 4). Inattention was not correlated to any of the EF measures (Table 4). More severe hyperactivity symptoms were also related to more misses (impulsive errors) during sustained attention (Table 4). Regression analysis, entering these three EF measures as predictors in a model with Hyperactivity as dependent, resulted in a significant model with R^2 =.189 (Table 5). A regression model with Impulsivity as dependent and the three EFs as predictors resulted in a significant model with R^2 =.129 (Table 5).

Table 4 ***Pearson correlations (1-tailed) of EF measures with ASD and ADHD symptom severity***

		ASD total	Social interaction	Communication	Repetitive Behavior	ADHD total	Inattention	Hyperactivity	Impulsivity	Age	Full scale IQ
Reaction Speed	Speed	.104	.182	.015	.121	-.042	-.026	-.001	-.087	.119	-.354**
	Fluctuation	.133	.190	.101	.129	.128	.090	.059	.179	.459**	-.295*
Sustained Attention	Tempo	-.096	-.035	-.090	-.160	-.115	-.087	-.141	-.050	.353**	-.543**
	Fluctuation	-.031	.016	-.025	-.093	-.204	-.145	-.209	-.159	.235*	-.548**
	Misses	.212	.143	.193	.169	.182	.086	.243*	.202	-.006	-.177
Attentional Flexibility RT[1]		.255*	.295*	.210	.075	.030	.059	-.012	-.039	.086	.130
Attentional Flexibility PE[2]		-.167	-.115	-.248*	-.016	-.012	-.068	.045	.097	.164	-.181
Inhibition RT[1]		.261*	.273*	.267*	-.015	.008	-.008	-.007	.066	.139	.074
Inhibition PE[2]		-.183	-.174	-.213	-.045	-.004	-.065	.079	.083	.184	-.087
Working Memory RT[3]		.146	.203	.116	-.084	-.080	-.094	-.051	.015	.145	-.236*
Working MemoryNM[3]		-.124	-.083	-.065	-.200	-.206	-.069	-.335**	-.254*	-.030	-.249*
Distraction RT[4]		.170	.096	.290*	.144	.217	.086	.277*	.298**	-.116	-.063
Distraction PF[4]		.162	.108	.125	.123	.106	.057	.069	.175	.096	-.067
Planning		.106	.128	.113	-.066	.100	.010	.158	.191	.601**	-.431**
Age		-.026	.038	-.031	-.212	-.117	-.109	-.202	.069	-	-.268*
Full Scale IQ		-.149	-.162	-.092	-.084	-.017	-.052	-.143	-.058	-	-

**Correlation is significant at the 0.01 level (1-tailed),*Correlation is significant at the 0.05 level (1-tailed) [1]Denotes decrease in speed when respectively flexibility or inhibition is required. [2] Denotes decrease in accuracy when respectively flexibility or inhibition is required, [3] Denotes decrease in speed (RT) or accuracy (NM) when memory load increased, [4]Denotes decrease in speed (RT) or accuracy (PF) when distraction is present.

Table 5 ***Regression ADHD severity***

Hyperactivity				
	F(df)	R^2	β	p
	3.883(3,50)	.189		.014
(constant)				.000
Sustained Attention[1]			.202	.123
Working Memory[2]			-.264	.047
Distraction[3]			.205	.121

Impulsivity				
	F(df)	R^2	β	p
	3.769(2,51)	.129		.030
(constant)				.000
Working Memory[2]			-.203	.133
Distraction[3]			.262	.054

[1]Denotes number of misses during sustained attention [2]Denotes decrease in accuracy when memory load increased [3] Denotes decrease in speed (RT) when distraction is present

Discussion

This study investigated executive functioning (EF) in subjects with 22q11DS and examined whether EF is related to the severity of ASD and ADHD symptoms. The use of an extensive battery of EF tasks allowed to generate a detailed profiles of executive dysfunctions, reflected in processing speed, stability and/or accuracy. We found less accurate responses when task demands required cognitive flexibility, resistance against distraction, inhibition or working memory capacity. Poorer alertness was reflected in slower reaction times and larger fluctuations in reaction speed. There were also deficits in sustained attention, as reflected in a higher fluctuation in tempo and a higher miss rate, the latter result indicates a decreased ability to maintain inhibitory control during time-on-task. Furthermore, planning skills were below average. We found that severity of ASD symptoms was correlated to poorer cognitive flexibility, inhibition and distractibility, while ADHD symptoms were found to be related to poorer quality of sustained attention and higher distractibility.
The majority of EF deficits were reflected in accuracy and not in reaction time. This finding is in line with the findings of Gur *et al.* (2014) but partly contradicts the results of Campbell *et al.* (2010), who did not find a difference in accuracy of performances on a mental flexibility task between 22q11DS and siblings. However, they also found poorer inhibition, planning skills and working memory capacity in individuals with 22q11DS (Campbell *et al.* 2010). Both studies are complementary in that findings give reason to believe that specific EF deficits, mostly reflected in lower accuracy, are present in 22q11DS.

As argued before, deficits in executive functions are believed to underlie behavioral and emotional problems and these deficits are possible developmental signs of vulnerability to more severe ASD and ADHD symptoms. The current study showed that decreases in tempo when cognitive flexibility or inhibition was required were related to ASD symptom severity. Focusing on detailed levels of ASD symptoms, a similar relation was found with severity of problems in reciprocal social interaction. Decreases in speed when inhibition and resistance to distraction were required were related to severity of impairment in communication. An increase in accuracy when flexibility was required was also related to a more severe impairment in communication. Together these results suggest that children with more severe autism symptoms decrease their tempo during complex tasks which allows them to perform relatively more accurately.

With respect to ADHD symptoms, severity of hyperactivity was related to poorer inhibition during sustained attention, higher distractibility and an increase in accuracy when memory load increased. Severity of impulsivity was related to higher distractibility and an increase in accuracy when memory load increased. This indicates that children with more ADHD symptoms do have problems with inhibition of responses and are easily distracted. However, when a higher demand is imposed on their working memory capacities, forcing them to focus on the task and be less easily distracted, individuals with more hyperactive or impulsive behavior seem to perform relatively better.

Interestingly, the relations between EF and ASD or ADHD partly seem to differ from findings in clinical groups with ASD and ADHD without 22q11DS. In children with ADHD, impairments in working memory and inhibitory control have been reported (Barkley 1997; Sonuga-Barke 2003), while in the current study inhibitory control was not associated with severity of ADHD symptoms in children with 22q11DS. This finding suggest a preliminary support of the idea of different neurobiological pathways, also on a neuropsychological level, leading to ADHD symptomatology as proposed by Durston and colleagues (Durston *et al.* 2011; De Zeeuw *et al.* 2012).

In children with idiopathic ASD deficits have been found in planning, inhibition and cognitive flexibility (Robinson *et al.* 2009; Ozonoff *et al.*1991). In the current study deficits in inhibition, flexibility and distractibility were related to severity of ASD symptoms but so far poor distractibility has not been reported in children with ASD. Our findings therefore suggest that in children with 22q11DS partly comparable EF deficits seem to influence the severity of ASD symptoms as compared to children with idiopathic ASD. These differences in findings may be explained by the fact that the current study investigated children who shared the same genetic etiology (22q11DS) whereas studies on idiopathic ASD or ADHD examine - by definition - samples of children with unknown genetic etiologies (Bruining *et al.* 2010), although heterogeneity in methods, i.e. the use of different tasks measuring the same constructs may also explain part of the differences in findings.

Age was found to be related to quality of EF. Older children demonstrated poorer sustained attention and planning skills than younger children. This outcome contradicts findings of others who found more pronounced impairments of EF in younger children with 22q11DS (Stoddard *et al.* 2011; Antshel *et al.* 2010), but is in line with the decline with age in the more general measures of cognitive functioning

(e.g. intelligence assessment, learning and memory) reported by Antshel *et al.* (2010). It is important to notice that inconsistencies between studies may be partly explained by the use of different EF concepts across studies and the use of general measures of cognitive functioning instead of detailed EFs.
It is important to replicate findings in a larger sample to disentangle the relation between behavioral and social problems involved in ASD and ADHD and EF in 22q11DS. The outcome of the current study suggests a relation between specific EF deficits and severity of both ASD and ADHD symptoms with medium to large effect sizes, thereby providing a helpful starting point for future research and the development of cognitive interventions. Because of the role of age emerging from this study, future research should be designed longitudinally.
The use of an extensive evaluation of EF and the investigation of EF in relation to ASD and ADHD separately are considered strengths of this study. There are also limitations.
The sample size can be considered relatively large for a study of individuals with a specific genetic disorder, but for some analyses the sample size was relatively small because data were not available for all cases on all measures. This complicates the generalization of the findings to the 22q11DS population, especially because of the large variability within the population. Results therefore need to be interpreted with caution. One may also argue that the lack of a control group can be seen as another limitation. The z-scores that were entered in the analyses are the results of computations, based on nonlinear regression functions that describe the relation between test age and task performance. These functions are fully implemented in the ANT program, based on norm samples varying in size between 3,100 to 6,700 subjects, depending on the task (De Sonneville 2014), and therefore considered to be reliable estimates of performance level. In addition, we think it is very difficult to determine what can be seen as an appropriate control group and whether such a control group should be matched on age, intelligence, developmental age or on other characteristic that makes this group unique by its syndrome specific features. Lastly, it needs to be mentioned that the Rey Complex Figure is not only a measure of planning abilities. Besides planning, the copy score of the RCFT also depends on the quality of other cognitive processes including visuoperceptual, visuocontructional and graphomotor skills (Straus *et al.* 2006). Although our findings are in line with previous studies that investigated planning using other measures, our results need to be interpreted with caution.

Conclusions

With this study we provided a detailed profile of impairments in EF experienced by a sample of children with 22q11DS. Some evidence has been found that the degree of impairment on specific EFs is related to the severity of ASD and ADHD symptoms in children with the syndrome. These results may help in defining the mediating role of neurocognitive dysfunctions in the development of social and behavioral problems in 22q11DS. Although it is not yet clear how this relation can be interpreted in a developmental perspective, it provides even more reason to monitor the development of individuals with 22q11DS carefully. At the same time this knowledge may help to develop cognitive interventions or adjust interventions to the needs of these children.

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Chapter 4

Facial emotion processing and its relation to autism and ADHD symptomatology in 22q11.2 Deletion Syndrome

Hidding, E., de Sonneville, L. M. J., van Engeland, H., Vorstman, J. A. S., & Swaab, H. Facial emotion processing and its relation to autism and ADHD symptomatology in 22q11.2 Deletion Syndrome. *Revised manuscript under review.*

Abstract

Children with 22q11.2 deletion syndrome (22q11DS) display symptoms of autism spectrum disorder (ASD) and/or attention-deficit-hyperactive disorder (ADHD). We examined whether problems in visual social information processing are related to these symptoms in 22q11DS.

Face-, facial emotion recognition and processing of abstract visuospatial information was evaluated in 45 children with 22q11DS. Relations with ASD and ADHD symptom severity were explored.

Slower, less accurate social information processing and less accurate abstract visuospatial information processing were found in children with 22q11DS. Less accurate processing of facial emotions and visuospatial information were related to more severe symptomatology.

Impairments in processing of social information may be part of a specific endophenotype of 22q11DS. Findings suggest these impairments to be possible underlying mechanisms of ASD/ADHD symptomatology.

Introduction

Children with 22q11.2 deletion syndrome (22q11DS) are at high risk to develop social problems that affect their daily functioning. Social-cognitive impairments in these children have been reported to result in social behavior problems that are part of the two major developmental disorders; autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD). Elevated rates of both disorders have been reported in 22q11DS (Schneider *et al.* 2014; Baker and Vorstman 2012; Green *et al.* 2009; Jolin *et al.* 2009; Niklasson *et al.* 2009; Vorstman et al. 2006). The quality of social cognitive abilities influences the competence in perceiving, interpreting and reacting adequately to emotions and behaviors of others (Green *et al.* 2005). Therefore, a better understanding of the relation between the quality of face and emotion recognition and the level of social impairment in individuals with 22q11DS may help to clarify the mechanisms underlying the behavioral disturbances in the social domain that are often found in 22q11DS.

The ability to correctly identify faces and their emotional states is considered to be essential in social functioning. Bruce and Young (1986) already argued that faces provide core social information for different purposes, in particular recognition of individuals and perception of emotional states (Bruce and Young 1998; Hole and Bourne 2010). However, since face and facial emotion recognition inevitably involves processing of visuospatial information, it is also important to investigate this skill of its own accord. Faces are thought to be processed on the basis of their configural organization while processing of abstract visuospatial information requires featural processing as a clear organizational structure is lacking (Hole and Bourne 2010; De Sonneville *et al.* 2002). Configural processing refers to the perception of relations among the features of a stimulus such as a face, in that the face can be seen as a meaningful whole. Featural processing is the opposite in which elements are processed piecemeal (Maurer *et al.* 2002). Recognition of facial emotions relies predominantly on configural face processing but may also be achieved through featural information processing, although this is less efficient and slower (Hole and Bourne 2010). Therefore, in order to increase insight into facial emotion processing in individuals with 22q11DS both configural and featural processing abilities need to be assessed.

Only a limited number of studies investigated face and emotion recognition in 22q11DS. Poorer accuracy of face recognition and emotion recognition has been found in comparison to healthy siblings, children with William syndrome (IQ matched) and typical controls (Campbell *et al.* 2009; Lajiness-O'Neill *et al.* 2005; Glaser *et al.* 2010; Campbell *et al.* 2010; Campbell *et al.* 2011; McCabe *et al.* 2011; Gur *et al.* 2014). In

addition, reduced tempo in remembering faces and identification of emotions has been reported in patients with 22q11DS (Gur *et al.* 2014). Accuracy of face recognition was reduced in both individuals with 22q11DS and children with idiopathic developmental delay as compared to normal control subjects (Glaser *et al.* 2010). This study also focused on the nature of face processing impairments in 22q11DS by using tasks that required featural or configural processing, respectively. Both the 22q11DS children and the children with idiopathic developmental delay displayed less accurate featural information processing compared to normal control subjects. Interestingly, the 22q11DS group also showed a decreased accuracy in configural processing, suggesting a specific impairment in visual facial processing in 22q11DS.
In studies comparing gender and age matched control subjects to individuals with 22q11DS, the 22q11DS group displayed more difficulties in identifying the facial emotions anger, disgust and fear, and also in the recognition of neutral faces (McCabe *et al.* 2011; Campbell *et al.* 2010). Jalbrzikowski *et al.* (2012) reported similar impairments, although in their study the identification of facial expressions of happiness, anger and sadness was most impaired in young adolescents with 22q11DS. Using an eyetracker, Campbell *et al.* (2010) reported atypical visual scanpath patterns in subjects with 22q11DS during facial emotion processing, compared to healthy controls. Individuals with 22q11DS spent more time on the mouth region and less on features that are important for accurate identification of emotions such as the eyes. This was also found during neutral face processing (Glaser *et al.* 2010), suggesting that individuals with 22q11DS have less adequate visual social information processing skills compared to control subjects. Only one eyetracking study compared scanpath patterns obtained during emotion recognition and during recognition of non-social stimuli (weather scene tasks) in 22q11DS (McCabe *et al.* 2011), showing that the patterns of adolescents with 22q11DS differed from those of control subjects, during processing of faces as well as processing of non-social visual stimuli. These results suggest that there may be a general visual information processing deficit besides the specific difficulties with processing of faces (McCabe *et al.* 2011).
In sum, studies thus far present evidence for less accurate visual face and emotion recognition and problems with visuospatial information in general in individuals with 22q11DS. Because of the known high risk for ASD and ADHD symptomatology in 22q11DS, it is clearly of interest to investigate whether abnormalities in visual social information processing are associated with the frequently observed symptoms in the social behavioral domain in 22q11DS. Thus far, little is known about deficits in face and facial emotion processing in subjects with 22q11DS and its relation with ASD and ADHD symptomatology. The few studies comparing face and facial emotion processing between subjects with 22q11DS and subjects with idiopathic autism, reported similar problems for both groups in memory for faces and accuracy of recognition of facial

emotions and non-social stimuli (McCabe *et al.* 2013; Lajiness-O'Neill *et al.* 2005). Patients with ASD and 22q11DS showed partly comparable patterns of scanpaths and deficits in emotion recognition, but subjects with 22q11DS took even less time looking at salient regions and spent more time looking at the mouth compared to subjects with ASD. Despite 22q11DS sharing phenotypical characteristics with ASD such as poorer facial emotion recognition, the underlying pathways of information processing might differ (McCabe *et al.* 2013). The identification of specific impairments in the processing of visuospatial information, differentiating between social and abstract visuospatial content, and elucidating their possible relation to ASD and ADHD symptomatology may help to improve our understanding of the neurodevelopmental impairments observed in 22q11DS.

The purpose of our study was to examine face and facial emotion recognition in children with 22q11DS. To find out whether impairments in these social skills are (partly) explained by impairments in the processing of visuospatial information in general, we also included a task requiring the recognition of abstract visuospatial patterns, differentiating between configural and featural processing strategies. In line with previous studies we anticipate that face and facial emotion processing in individuals with 22q11DS is impaired. Here, we hypothesized that these impairments can at least partly be explained by impairments in general visual information processing. Lastly we hypothesized less well developed visual social information processing to be related to more severe ASD and ADHD symptomatology.

Method

In the present study, 27 females and 18 males with genetically confirmed 22q11DS participated (*M*age = 13.3, SD=2.7, range 9-18.5; Full scale intelligence: *M*= 66.3, SD=12.6) . The study was part of a nationwide study. Assessment took place at the Department of Psychiatry, Brain Center Rudolph Magnus of the University Medical Centre Utrecht (UMCU) and patients were recruited via the website and newsletter of the 22q11DS parents' network in the Netherlands or via referral by various medical services. Parents and participants were informed by phone about the aims of the study and received a complete description of the study in writing before they decided on participation. Informed consent was obtained from participants and parents or caretakers. The assessment protocol was approved by the Dutch Central Committee on Research Involving Human Subjects. Assessments took place at the outpatient center of the UMCU and were carried out by an experienced child neuropsychologist and child psychiatrist.

Measures

Severity of ASD and ADHD symptomatology

Psychiatric classifications were made according to DSM-IV criteria (American Psychiatric Association 2000) resulting from a multidisciplinary consensus meeting headed by an experienced child psychiatrist, on the basis of clinically structured and semi-structured interviews (with the child and the caregivers), observation of the child, questionnaires, and assessment of intellectual functioning. The assessment protocol has been described in a previously published study (Vorstman *et al.* 2006). An overview of the DSM-IV classifications of the sample, reflecting the multidisciplinary clinical consensus based on all available patient information, is provided by Table 1.

The assessment protocol included *the Autism Diagnostic Interview-Revised* (ADI-R; Rutter *et al.* 2003), scored by certified interviewers, used to quantify autistic symptoms. The ADI-R provided scores for the three domains in which children with autism spectrum disorders (ASD) experience difficulties, i.e. reciprocal social interaction, communication impairment, repetitive and stereotyped behaviors. The classifications autism and pervasive developmental disorder not otherwise specified are both referred to as ASD.

In some cases, the DSM-IV diagnoses deviate from the classifications that would be obtained if only the outcomes of the questionnaires were used. According to DSM-IV guidelines, a diagnosis of both ADHD and ASD in one individual is not allowed (American Psychiatric Association 2000). In those cases in which the ASD symptomatology was more dominantly present explaining also the ADHD symptoms, no (additional) ADHD diagnosis was made based on such symptoms. As a consequence, only one individual was diagnosed with ADHD comorbid to an ASD diagnosis because this ASD diagnosis could not explain the severely comorbid ADHD symptomatology (Table 1).

Because of the high prevalence of both ASD and ADHD in 22q11DS the possible co-occurrence of symptoms of both disorders was also investigated. To this end, we used the three ADHD domains (inattention, hyperactivity, impulsivity), as rated with a structured questionnaire based on the criteria of DSM-IV as a measure of severity of ADHD symptoms. This questionnaire consisted of comparable items as the Conners' Rating Scales-Revised (Conners 1997) and the Dutch version of the ADHD DSM-IV rating scale (Kooij *et al.* 2008). Likewise, the three domains of the ADI-R were used as a measure of severity autism symptoms. Table 2 provides the means and distribution of the ASD and ADHD severity scores.

Table 1 ***Psychiatric classifications according to DSM-IV criteria with primary diagnoses and comorbid diagnoses.***

Diagnostic classification (primary)		*Comorbid diagnoses***			
	N	ASD	ADHD	Dep.dis	ODD*	Psych.dis
Autism spectrum disorder (ASD)	25		1	3		4
Attention Deficit Hyperactivity Disorder	0					
Anxiety Disorder	0					
Conversion Disorder	1					
Depressive disorder (Dep.dis)	2					
Psychotic disorder (Psych.dis)	1					
Without psychiatric classification	16					
Total	45		1	3		4

* Oppositional defiant disorder
** Represent comorbid diagnoses within the total N of 45

Table 2 ***Autism and ADHD severity scores.***

	N	*M*	*SD*	Range
ADHD-total	45	12.3	8.7	0-30
Inattention	45	8.4	6.4	0-23
Hyperactivity	45	1.9	2.4	0-7
Impulsivity	45	1.9	2.0	0-9
ADI-total	46	25.7	13.9	0-49
Reciprocal social interaction	46	11.5	7.1	0-26
Communication impairment	46	8.2	5.4	0-19
Repetitive and stereotyped behaviors	46	2.2	2.0	0-8

Intellectual functioning was assed using the Dutch version of the Wechsler Intelligence Scales for Children WISC-III (Wechsler 2002; Wechsler 2005b). In one case the WISC-R was used (Wechsler 1974), in four cases the adult scale (WAIS-III; Wechsler 2005a) for adolescents older than 16 years was applied. In one case information about intelligence was missing.

Visual information processing was assessed with the use of the Amsterdam Neuropsychological Tasks (ANT) program (De Sonneville, 1999; 2005). Test-retest

reliability, construct-, criterion, and discriminant validity of the computerized ANT-tasks are satisfactory and have extensively been described elsewhere (De Sonneville 2014; Gunther *et al.* 2005; Rowbotham *et al.* 2009; Huijbregts *et al.* 2002). The ANT tasks, used in this study, will be briefly described, for detailed descriptions including examples of signals and timing between signals, see e.g. De Sonneville *et al.* (2002).

Face recognition (FR) With this task speed and accuracy of recognizing (neutral) faces was measured. From a set of 20 pictures of different persons (boys, girls, men and women) a probe, the to-be-recognized face, is presented on a monitor for 2.5 seconds, prior to the imperative signal which consists of four digitized high-quality color photos of human faces. Gender and age category (children, adults) of signal and probe always match. A 'yes'- response is required when the probe is present (20 trials) by pressing the mouse button below the index finger of the preferred hand, and a 'no'- response when the probe (20 trials) is not present, by pressing the mouse key below the index finger of the non-preferred hand. Main outcome variables were mean reaction time and number of errors.

Identification of Facial Emotions (IFE) This task examined the ability to identify emotions from facial expression. Participants were asked to judge whether a face showed a specific expression by pressing the 'yes'- key or another non target emotion by pressing the 'no'- key.
The total stimulus set consisted of 32 pictures from four different persons, each showing the eight emotions: happy, sad, anger, fear, disgust, surprise, shame, and contempt. The task consists of eight parts of 40 trials in which half of the trials contain the target emotion, whereas in the other half a random selection of the other emotions is presented. Four task parts were administered to measure the recognition of the basic emotions happy, sad, anger, and fear, respectively. Main outcome variables were mean reaction time and number of errors per task part.

Feature Identification (FI) This pattern recognition task assesses speed and accuracy of processing abstract visuospatial information. Subjects were asked to detect a predefined target pattern in a signal consisting of four patterns. The subject was asked to press the 'yes'-key when the pattern was present (half of the signals, 40 trials) and the 'no'-key when the pattern was not present. Two different task conditions made it possible to discriminate between featural and configural processing strategies. In the 'similar' condition, the distractor patterns looked very similar to the target pattern, inducing a featural processing strategy to detect the target. In the 'dissimilar' condition (other half of the signals) the distractors were very dissimilar to the target

signal, invoking a configural processing strategy. Mean reaction time and number of errors were obtained for the similar and dissimilar conditions separately.

Statistical analyses

Main outcome parameters for analyses are z-scores, which are automatically computed by means of nonlinear regression functions that describe the relation between test age and task performance. These functions are fully implemented in the ANT program and based on norm samples varying in size between 3,100 to 6,700 subjects, depending on the task (De Sonneville, 2014), and are therefore considered to be reliable estimates of performance level. Results were examined for extreme values. As extreme values are a clinical reality in this population, z-scores ≥ 6 were set to 6 to keep these subjects in the analyses. One subject with an error rate >50% was excluded from statistical analysis as this rate is worse than chance level. In addition, missing values in the final sample are the consequence of an inability of the subject to complete difficult task parts, or skipping parts because of running out of time. As a result, degrees of freedom will slightly vary between analyses.

Comparison to the norm

To determine whether mean performance of the subjects with 22q11DS differed from the norm, i.e. differed from zero for z-scores, the intercept test of the multiple analyses of variance (MANOVA) was used. Results were evaluated per task by MANOVA, with the z-scores for speed (reaction time (RT)) and accuracy (percentage of errors) as dependent factors. In case the multivariate test was significant, the univariate results were presented as well.

Within-subject comparisons

Task conditions were used as levels of within-subject (WS) factors in repeated measures ANOVAs with speed and accuracy of performance as dependent variables respectively.

A significant WS factor effect implies that differences in performance level between the group and the norm depends on WS factor level (interaction). Faces present complex, but organized concrete visuospatial patterns. By contrasting the results of the similar and dissimilar condition of task FI it can be determined whether type of processing (featural vs. configural) differentiates children with 22q11DS from the norm. By contrasting the results of task FR and task FI it can be determined whether processing of facial information rather than processing of abstract visuospatial information (or vice versa) differentiates children with 22q11DS from the norm. Similarly, by contrasting the results of task FR and IFE, it can be determined whether

processing of facial emotions rather than processing of faces (or vice versa) differentiates children with 22q11DS from the norm.
WS factors per task were: *Signal* (similar vs. dissimilar) for task FI, and *Emotion* (positive vs. negative emotion - to reduce the number of analyses, it was decided to lump the three negative emotions together). When contrasting results across tasks, the following WS factors were used: *Pattern* (patterns vs. faces) for task FI and FR with separate contrasts for the similar and dissimilar condition of task FI, and *Facial Information* (neutral faces vs. facial emotions) for task FR and IFE.

Severity of ASD and ADHD symptomatology

Pearson correlations were calculated for the relation between severity of ASD and ADHD symptoms and visual social information processing (small effect size: r=0.1-0.23; medium: r=0.24-0.36; large: r≥ .37;Cohen 1992). To limit multiple testing, total symptom severity scores were used for ASD and ADHD separately.
Correlations between quality of featural information processing and symptom severity were also calculated. For the correlation analyses *Quality of featural processing* was operationalized as the difference of the similar condition score minus the dissimilar condition score. A high difference indicated poorer (slower/less accurate) featural processing. The role of Full Scale Intelligence (FSIQ) as a possible covariate was investigated.

Results

Standardized means of total group performances on all tasks of visual information processing are presented in Figure 1. Negative deviations from zero indicate more efficient performances, while positive deviations reflect worse performances.

Feature identification

Participants were less accurate, but not slower than the norm, as was shown by a significant multivariate effect for the identification of patterns [$F(4,35) = 9.162$, $p<.001$, $\eta_p^2 = .511$] and univariate results revealing significant effects of accuracy in the dissimilar [$F(1,38) = 7.226$, $p=.011$, $\eta_p^2 = .160$] and similar condition [$F(1,38) = 20.114$, $p<.001$, $\eta_p^2 = .346$], but not for speed in both conditions ($.154<p<.469$). Children with 22q11DS compared to the norm performed worst in the similar condition, reflecting difficulties in featural processing as was indicated by a significant effect of the WS

factor Signal for accuracy of processing [F(1,39)=7.612, p=.009, η_p^2 =.163]. On speed no significant effect of Signal was found (p=.279).

Face Recognition

Subjects with 22q11DS were slower [F(1,40) = 23.178, p<.0001, η_p^2 =.367] and less accurate in the recognition of faces [F(1,40) = 83.361, p<.0001, η_p^2 =.676] as compared to the norm (multivariate effect [F(2,39) = 54.631, p<.0001, η_p^2 =.737]).

Emotion Recognition

A significant multivariate effect of Emotion Recognition was found [F(4,39) = 31.372, p<.001, η_p^2 =.763]. Participants were slower and less accurate on emotion recognition compared to the norm as was demonstrated by significant univariate results for the accuracy of processing positive emotions (happy) [F(1,42) = 8.085, p=.007, η_p^2 =.161], negative emotions [F(1,42) = 123.087, p<.001, η_p^2 =.746] as well as on speed of processing positive emotions[F(1,42) = 44.951, p<.001, η_p^2 =.517] and negative emotions [F(1,42) = 21.114, p<.001, η_p^2 =.335]. When comparing the quality of recognition of positive versus negative emotions (WS factor Emotion), no significant difference was found on speed (p=.089), but a significant effect was found on accuracy [F(1,42)=56.892, p<.001, η_p^2 =.575], indicating that the children with 22q11DS as compared to the norm performed worst on the recognition of negative emotions.

Face recognition vs. Feature identification

The WS factor Pattern (faces vs. features) was significant on accuracy of processing [F(2,76) = 5.456, p=.006, η_p^2 =.126], but not on speed (p=.121). WS contrast (faces vs. dissimilar patterns) revealed that accuracy of face recognition was significantly worse compared to the accuracy on dissimilar patterns [F(1,37) = 9.423, p=.004, η_p^2 =.203], while no significant difference was found between accuracy of face recognition vs. similar patterns (p=.938).

Emotion recognition vs. Face Recognition

The WS factor Facial Information (Face vs. Emotions) was significant on accuracy of processing [$F(2,76) = 28.000$, $p<.0001$, $\eta_p^2 = .424$], but not on speed ($p=.582$). WS contrasts revealed that the accuracy of recognizing positive emotions was significantly better than the accuracy of face recognition [$F(1,38) = 39.173$, $p<.0001$, $\eta_p^2 = .508$] in the children with 22q11DS as compared to the norm. No significant difference in accuracy of processing negative emotions as compared to faces was found ($p=.778$).

Severity of ASD and ADHD symptomatology

Based on the findings, we decided to only include accuracy scores in the correlational analyses. FSIQ was related to accuracy of processing of positive and negative emotions (Table 3), children with a lower FSIQ showed more difficulties with accurate processing of emotions. No correlations were found between FSIQ and the other measures.

Regarding *Quality of featural processing* (similar minus dissimilar scores), correlations were found between accuracy of processing and severity of ADHD and ASD symptoms (Table 3). This indicates that children with less well developed featural processing skills, showed also more severe ADHD and ASD symptomatology.
Accuracy of facial emotion recognition was correlated with ASD symptomatology and accuracy of negative emotion recognition was related to ADHD symptomatology (Table 3). This indicates that children who display more difficulties with emotion processing also show more ASD and ADHD symptomatology.
Using FSIQ as a covariate, these effects remained significant for the relation between negative emotion processing and severity of symptoms (Table 3).
Using quality of featural processing as covariate removed the effect of emotion recognition and ADHD symptomatology, while the effect for ASD symptomatology remained significant (Table 3).

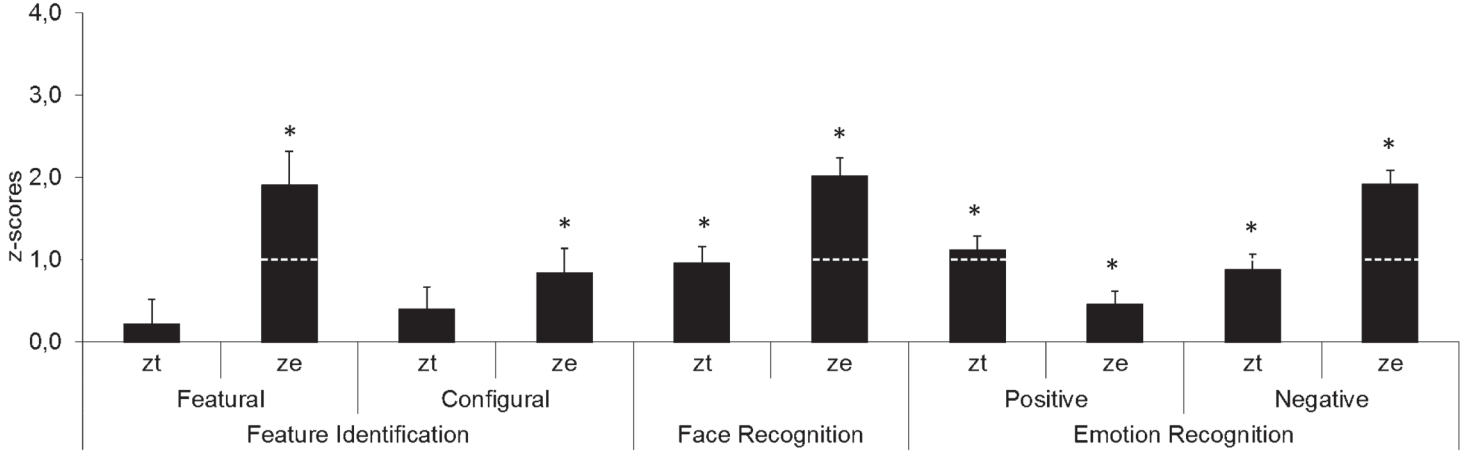

Figure 1 Mean z-scores of the total group on Feature Identification, Face Recognition and Emotion Recognition. With scores for speed (zt) and accuracy (ze) of performances. * Significant at p<0.01.

Table 3 ***Pearson and partial correlations of the accuracy scores with ASD and ADHD symptom severity***

	ASD-total	ADHD-total	FSIQ
Pearson correlations			
Quality of Featural processing	.286*	.272*	-.115
Emotion recognition (negative)	.361**	.302*	-.512**
Emotion recognition (positive)	.302*	.038	-.281*
Face Recognition	-.006	.039	-.232
Partial controlling correlations for FSIQ			
Quality of Featural processing	.270*	.273*	-
Emotion recognition (negative)	.296*	.345*	-
Emotion recognition (positive)	.256*	.037	-
Partial correlations controlling for featural processing			
Emotion recognition (negative)	.273*	.211	-
Emotion recognition (positive)	.272*	-.004	-

**Correlation is significant at the 0.01 level (1-tailed),*Correlation is significant at the 0.05 level (1-tailed)

Discussion

The purpose of our study was to investigate whether face and facial emotion recognition in children with 22q11DS is impaired and to find out whether these impairments are (partly) due to impairments in processing of visuospatial information in general. Secondly, we aimed to investigate whether identified deficits are related to severity of ASD and ADHD symptomatology.
Outcomes revealed impairments in both face and emotion recognition in subjects with 22q11DS as compared to the norm. More severe difficulties were found in recognizing negative emotions compared to positive emotions. Processing of abstract visual information was also impaired, with individuals with 22q11DS experiencing more severe impairments in featural processing of information as compared to configural processing. Processing of facial information was more severely impaired as compared to processing of abstract visual information, although no difference was found between face processing and featural processing of abstract information, suggesting that children with 22q11DS experience difficulties in the processing of complex abstract and social visual information.

Our finding of impairments in accuracy of face processing are in line with previous findings (Campbell *et al.* 2009; Lajiness-O'Neill *et al.* 2005; Glaser *et al.* 2010). We add to these results by showing that individuals with 22q11DS are also slower in processing of facial information. Because we were interested in possible face-specific deficits in visual information processing, we contrasted processing of facial information with processing of abstract visuospatial patterns, while differentiating between featural and configural processing strategies. Our results show impairments in both types of processing with featural information processing most affected, which is in line with the findings of Glaser *et al.* (2010) who found impaired featural processing of social stimuli. However, the current study gives reason to believe that this deficit in social information processing may at least partly originate from a general impairment in the processing of visuospatial information. Although processing of facial information was weaker as compared to the processing of abstract visuospatial information, comparable levels of impairments in accuracy of face recognition and processing of abstract visuospatial patterns that require featural processing were found. This could indicate that the difficulties with featural processing result in poorer processing of facial information or, alternatively, suggests that individuals with 22q11DS process faces by using a featural rather than configural strategy, which is known to be less adequate and slower (Hole and Bourne 2010).
The comparison of face recognition and the recognition of facial emotions resulted in similar levels of problems for the recognition of negative emotions but relatively less difficulties for the recognition of positive emotions. Possibly, recognition of positive emotions is relatively less influenced by a deficit in featural processing of information, as a laughing mouth stands out as a salient characteristic that can be best processed in a fast configural way. Moreover, previous studies showed that children with 22q11DS spend relatively more time looking at the mouth when processing faces (Campbell *et al.* 2010; Glaser *et al.* 2010). For positive emotion recognition the mouth area is necessary and sufficient for accurate identification while for the identification of negative emotions it is also critical to look at other features of the face, for example at the eye-brow (Beaudry *et al.* 2014; Calvo and Nummenmaa 2008).
Another aim of our study was to investigate the relation between the quality of visual social information processing and severity of ASD and ADHD symptomatology. The ability to correctly recognize faces and facial emotions is important for social behavior and deficits in this ability are possibly developmental signs of vulnerability to more social behavioral problems that are common in ASD and ADHD. We found accuracy of recognition of negative emotions to be related to severity of ASD and ADHD symptomatology. This is in line with the specific deficits in face and facial emotion processing that are found in individuals with idiopathic ASD or ADHD (Singh *et al.* 1998; Njiokiktjien *et al.* 2001; Serra *et al.* 2003; Deruelle *et al.* 2004; Yuill and Lyon

2007; Herba *et al.* 2008; Shin *et al.* 2008; Sinzig *et al.* 2008; Williams *et al.* 2008; Hole and Bourne 2010; Oerlemans *et al.* 2014). Given the deficit in processing of abstract visuospatial information which possibly underlies the deficient facial information processing, we also investigated the relation between abstract visuospatial information processing and ASD and ADHD symptomatology. Children with poorer featural processing of abstract visuospatial information showed also more ASD and ADHD symptomatology. Remarkably, when using quality of featural processing as covariate, the relation between emotion processing and severity of ADHD symptomatology no longer exists. This could indicate that in individuals with 22q11DS different mechanisms are involved in the development of social behavioral problems as compared to individuals with idiopathic ASD and ADHD symptomatology, indicating specific problems in featural processing in 22q11DS.

Although this finding needs to be replicated in a larger sample, it supports the idea of different neurobiological pathways leading to the social behavioral problems reported in developmental disorders like ASD and ADHD (Durston *et al.* 2011; De Zeeuw *et al.* 2012). Possibly, these differences in developmental pathways are the consequence of the involvement of different genetic etiology (Bruining *et al.* 2010).

The current study adds to the literature by detailed evaluation of visuospatial information processing in 22q11DS using tasks with low demands and that require less cognitive flexibility as compared to tasks in other studies. Studies comparing general visuospatial information processing and face and facial emotion processing are scarce. Therefore, the use of separate tasks for face recognition, emotion recognition, and the identification of abstract visuospatial stimuli differentiating between featural and configural processing in this study can be considered a strength. A limitation is the relatively small sample size which complicates the generalization of findings.

Conclusions

This study has shown that individuals with 22q11DS are impaired in face and facial emotion recognition as well as in processing of abstract visuospatial information. These impairments may be part of a specific endophenotype of 22q11DS. The finding that less adequate featural processing was related to more severe ASD and ADHD symptomatology, and especially that this explained the relation between quality of emotion processing and ADHD symptomatology, is important since it suggests that in 22q11DS specific mechanisms are involved in the development of ASD and ADHD symptoms as compared to idiopathic ASD and ADHD populations. However, more research into the role of visual social information processing in relation to ASD and ADHD symptomatology in larger samples is necessary.

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Chapter 5

The role of COMT and plasma proline in the variable penetrance of social deficits in 22q11.2 Deletion Syndrome

Hidding, E., Swaab, H., de Sonneville, L.M.J., van Engeland, H., & Vorstman, J.A.S. The Role of COMT and plasma proline in the variable penetrance of social deficits in 22q11.2 Deletion Syndrome. *Revised manuscript submitted.*

Abstract

This paper examines how $COMT^{158}$ genotypes and plasma proline levels are associated with variable penetrance of social behavioral and cognitive problems in 22q11.2 deletion syndrome (22q11DS).

Quality of social functioning of 45 participants with 22q11DS (27 females) with a mean age of 13.3 (SD =2.7, range 9-18.5) was assessed using the Autism Diagnostic Interview Revised. Quality of face and facial emotion processing was evaluated to examine social cognitive problems. Associations with $COMT^{158}$ genotypes and proline levels were examined.

High proline levels and poor face recognition in individuals with the $COMT^{MET}$ allele, together with poor facial emotion recognition, explained almost 50% of the variance in severity of autism symptomatology in individuals with 22q11DS.

High proline levels and a decreased capacity to break down dopamine as a result of the $COMT^{MET}$ variant are both relevant in the expression of the social phenotype in patients with 22q11DS. This epistatic interaction effect between the $COMT^{158}$ genotype and proline on the expression of social deficits in 22q11DS demonstrates how factors other than the direct effects of the deletion itself can modulate the penetrance of associated cognitive and behavioral outcomes. The findings of this study are not only relevant to our insight into 22q11DS, but also provide a model to better understand the phenomenon of variable penetrance in other pathogenic genetic variants.

Introduction

The 22q11.2 deletion syndrome (22q11DS) is characterized by a large variability in its phenotypic expression. The syndrome is associated with a high vulnerability to a variety of behavioral disorders with an onset in childhood or adolescence including anxiety disorders, mood disorders, attention deficit hyperactivity disorder (ADHD), oppositional defiant disorder, and autism spectrum disorders (ASDs) in 30 – 50% of affected individuals (Schneider *et al.* 2014; Jolin *et al.* 2009; Baker and Vorstman 2012; Niklasson *et al.* 2009; Vorstman *et al.* 2006; Jonas *et al.* 2014). Most important, the 22q11.2 deletion is the highest known single genetic risk factor for schizophrenia (Murphy *et al.* 1999, Schneider *et al.* 2014). The deletion affects approximately 45 genes, many of which are involved in the development and functioning of the brain (Meechan *et al.* 2011, Mehta *et al.* 2014, Dennis and Thompson 2013). The study of individuals with 22q11DS thus provides an exceptional opportunity to elucidate how genetic variation can affect brain development and how interaction with additional factors influence the manifestation of cognitive and behavioral outcomes. This knowledge may also be valuable for other recurrent copy number variants (CNVs) since almost all of them are associated with variable penetrance of different brain-related phenotypes (Girirajan and Eichler 2010). This variable penetrance of phenotypes in genetic disorders poses a formidable challenge for clinicians and at present its mechanisms are still not fully understood.

One of the domains in which children with 22q11DS experience difficulties is the social domain. Most studies consistently report social problems, both cognitive and behavioral, as well as repetitive behavioral patterns that are considered by some as characteristic for autism symptomatology (Schneider *et al.* 2014, Baker and Vorstman 2012, Niklasson *et al.* 2001, Fine *et al.* 2005). Investigating which factors (stochastic, additional genetic or environmental) influence the developmental pathways associated with the 22q11.2 deletion, such that one child develops social problems while another child does not, may further enhance our understanding of the variability in penetrance of phenotypic expression. Here, we propose to examine the influence of two additional factors that may modulate the high vulnerability to social cognitive and behavioral deficits in children with 22q11DS: the genotype of the remaining allele of COMT and plasma levels of the amino acid proline.

The gene COMT is hemizygously deleted in individuals with 22q11DS. This gene encodes Catechol-*O*-Methyltransferase, an enzyme involved in degradation of catecholamines, including dopamine (Philip and Bassett 2011; Williams 2011; Graf *et al.* 2001). A common polymorphism at codon 158 results in a decrease of COMT activity associated with the $COMT^{MET}$ variant (Chen *et al.* 2004; Graf *et al.* 2001; Jonas *et al.* 2014). In individuals with 22q11DS, the functional effects of this polymorphism may be increased since only one copy of the gene is present. It is hypothesized that individuals with 22q11DS and the $COMT^{MET}$ variant have a reduced capacity to eliminate dopamine, particularly in the prefrontal cortex (Simon *et al.* 2005). This could influence cognitive functioning, although findings in 22q11DS are inconsistent (Baker *et al.* 2005; Bearden *et al.* 2004; Campbell *et al.* 2010; Carmel *et al.* 2014; Furniss *et al.* 2011; Kates *et al.* 2006; Shapiro *et al.* 2014; Shashi *et al.* 2006). The $COMT^{158}$ polymorphism is associated with functioning of the prefrontal cortex which

is necessary for processing of social relevant information (Azuma 2015; Coman *et al.* 2010; Kempton *et al.* 2009). Effects of the COMT^{158} polymorphism on social cognition have been found in healthy subjects and patients with bipolar disorder (Lin *et al.* 2013; Soeiro-de-Souza *et al.* 2012; Weiss *et al.* 2007). However, thus far, no studies have investigated the relation between this polymorphism and social cognition in 22q11DS, even though abnormalities in this domain are reported often in patients with 22q11DS (e.g. Campbell *et al.* 2010; Campbell *et al.* 2009; Glaser *et al.* 2010; Gur *et al.* 2014; Jalbrzikowski *et al.* 2012).

Regarding social behavioral outcomes, the COMT^{MET} variant is found to be associated with an increased vulnerability to several behavioral disorders including ADHD and obsessive compulsive disorder (Gothelf *et al.* 2007). However, despite the high prevalence of social cognitive and behavioral problems associated with ASD in the syndrome, only one study investigated the relation between COMT gene expression and ASD (Radoeva *et al.* 2014). This study included the PRODH gene which encodes proline dehydrogenase that catalyzes the conversion of proline into glutamate. Given the importance of glutamate signaling in visual information processing, PRODH variation may affect the vulnerability to visual processing deficits in 22q11DS (Magnee *et al.* 2011). Proline influences the quality of visual information processing that is necessary to deal with social stimuli while dopaminergic dysregulation influences higher cognitive processes and social cognition that, when impaired, underlie the deficits in social functioning observed in children with autism (Herba *et al.* 2008; Rump *et al.* 2009). Findings of several studies indicate an epistatic interaction between COMT and PRODH, suggesting that the phenotypic effect of one genetic variant depends on the variation in another gene (Jonas *et al.* 2014; Paterlini *et al.* 2005; Raux *et al.* 2007). For example high proline levels have been found associated with impaired visual processing in individuals with the COMT^{MET} allele, but not in individuals with the COMT^{VAL} allele (Magnee *et al.* 2011). The same interaction was also found in an eye-movement study (Vorstman *et al.* 2009) and another study showed that hyperprolinemia in individuals with the COMT^{MET} allele was associated with the risk for psychosis (Raux *et al.* 2007). Recently, an epistatic interaction between COMT and PRODH genotypes on the probability of ASD was found in a group of individuals (aged 6-21 years) with 22q11DS (Radoeva *et al.* 2014).

Here, we propose to expand these findings by examining the possible interaction of the COMT^{158} genotype and variable plasma proline levels, which is the primary biological consequence of PRODH variation (Bender *et al.* 2005). Since social cognitive processes are involved in the emergence of social behavioral problems associated with ASD, we will study not only the effect of these factors on the risk of these social behavioral problems, but also on the child's capacity of face and facial emotion recognition. We expect the relation between COMT genotype and social behavioral problems to be dependent of, or influenced by plasma proline level. Since COMT genotypes have been previously found to be associated with cognitive functioning in 22q11DS, we also hypothesize an impact of COMT genotypes on social cognitive processes and explore the possibility of an interaction between impairments in social cognition and COMT genotypes.

Method

In the present study, 27 females and 18 males with genetically confirmed 22q11DS participated (*M*age = 13.3, SD=2.7, range 9-18.5; Full scale intelligence: *M*= 66.3, SD=12.6) . The study was part of a nationwide study. Assessments took place at the Department of Psychiatry, Brain Center Rudolph Magnus of the University Medical Centre Utrecht (UMCU) and were carried out by an experienced child neuropsychologist and child psychiatrist. Patients were recruited via the website and newsletter of the 22q11DS parents' network in the Netherlands or via referral by various medical services. Parents and participants were informed about the aims of the study and received a complete description of the study in writing before they decided on participation. Informed consent was obtained from participants and parents or caretakers. The assessment protocol was approved by the Dutch Central Committee on Research Involving Human Subjects.

Measures

Psychiatric classifications were made according to DSM-IV criteria (American Psychiatric Association 2000) resulting from a multidisciplinary consensus meeting headed by an experienced child psychiatrist. The assessment protocol has been described elsewhere (Hidding *et al.* 2015; Vorstman *et al.* 2006) and included *the Autism Diagnostic Interview-Revised* (ADI-R; Rutter *et al.* 2003), scored by certified interviewers. The ADI-R provided scores for the three domains in which children with autism spectrum disorders (ASD) experience difficulties, i.e. reciprocal social interaction, communication impairment, repetitive and stereotyped behaviors. These domains were used as a measure of severity social behavioral problems. Table 1 provides the means and distribution of the severity scores.

Table 1 ***Severity scores of social behavioral problems.***

	N	*M*	*SD*	Range
ADI-total	45	26.1	13.9	0-49
Reciprocal social interaction	45	11.6	7.2	0-26
Communication impairment	45	8.3	5.4	0-19
Repetitive and stereotyped behaviors	45	2.8	2.0	0-8

Social information processing

Social information processing was assessed with the use of the Amsterdam Neuropsychological Tasks (ANT) program (De Sonneville 1999, 2005). Test-retest reliability, construct-, criterion, and discriminant validity of the computerized ANT-tasks are satisfactory and have extensively been described elsewhere (De Sonneville 2014; Gunther *et al.* 2005; Huijbregts *et al.* 2002; Rowbotham *et al.* 2009). The ANT tasks, used in this study, will be briefly described, for detailed descriptions see e.g. De Sonneville *et al.* (2002).

Face recognition (FR) With this task speed and accuracy of recognizing (neutral) faces was measured. From a set of 20 pictures of different persons (boys, girls, men and women) a probe, the to-be-recognized face, is presented on a monitor for 2.5 seconds, prior to the imperative signal which consists of four digitized high-quality color photos of human faces. Gender and age category (children, adults) of signal and probe always match. A 'yes'- response is required when the probe is present (20 trials) by pressing the mouse button below the index finger of the preferred hand, and a 'no'-response when the probe (20 trials) is not present, by pressing the mouse key below the index finger of the non-preferred hand. Main outcome variables were mean reaction time and number of errors.

Identification of Facial Emotions (IFE) This task examined the ability to identify emotions from facial expression. Participants were asked to judge whether a face showed a specific expression by pressing the 'yes'- key or another non target emotion by pressing the 'no'- key.
The total stimulus set consisted of 32 pictures from four different persons, each showing the eight emotions: happy, sad, anger, fear, disgust, surprise, shame, and contempt. The task consists of eight parts of 40 trials in which half of the trials contain the target emotion, whereas in the other half a random selection of the other emotions is presented. Four task parts were administered to measure the recognition of the basic emotions happy, sad, anger, and fear, respectively. Main outcome variables were mean reaction time and number of errors per part. To reduce the number of analyses, it was decided to lump the results of the three negative parts together.

COMT158 genotyping and proline measurement

COMT*158* genotyping was carried out using allele-specific TaqMan probes (Applied Biosystems, Foster City, CA). Methodological details of PCR and sequence detection have been published in detail elsewhere (Vorstman *et al.* 2009).
Plasma proline levels were assessed by automated ion exchange chromatography with post-column ninhydrin derivatization, using JEOL AminoTac (JEOL AminoTac JLC-500/V, Tokyo, Japan) following AM blood draw. Methodological details of the plasma proline measurement protocol have also been published in detail elsewhere (Vorstman *et al.* 2009).

Statistical analyses

Main outcome parameters for analyses of the social information processing tasks are z-scores, which are automatically computed by means of nonlinear regression functions that describe the relation between test age and task performance. These functions are fully implemented in the ANT program and based on norm samples varying in size between 3,100 to 6,700 subjects, depending on the task (De Sonneville 2014), and are therefore considered to be reliable estimates of performance level. Results were examined for extreme values. As extreme values are a clinical reality in this population, z-scores ≥ 6 were set to 6 to keep these subjects in the analyses. One subject with an error rate >50% was excluded from statistical analysis as this rate is worse than chance level. In addition, missing values in the final sample are the consequence of an inability of the subject to complete difficult task parts, or skipping

parts because of running out of time. As a result, degrees of freedom will slightly vary between analyses.
Prior to analysis, normality of the data was examined using skewness and kurtosis measures and the Shapiro-Wilk tests (α=.01). Since the outcome parameter proline and two of the social information processing outcome parameters appeared to be skewed, Log transformations were applied to proline and all social information processing outcome parameters.
To examine the relation between severity of social behavioral problems and $COMT^{158}$ allele status as well as the influence of proline level, multiple regression analyses were performed with severity of social behavioral problems (separate analyses for sum score and scale scores) as dependent measures, $COMT^{158}$ allele status as fixed factor and proline level as covariate. Since we expect proline to interact with $COMT^{158}$ allele status, moderation analyses using the method of Aiken and West (1991) were performed to investigate the interaction between $COMT^{158}$ allele status and proline level.
To investigate the association of social cognition and severity of social behavioral problems as well as with $COMT^{158}$ allele status and proline levels, zero order correlations between social cognition (face and facial emotion recognition) and severity of autism symptoms were explored, followed by partial correlations with $COMT^{158}$ allele status and proline levels as covariates, respectively (small effect size: r = 0.1-0.23; medium: r = 0.24-0.36; large: $r \geq 0.37$; Cohen 1992).
Based on these exploratory correlational analyses, relevant social cognition parameters were included in moderation analyses with social behavioral problems as dependent measures, social cognition as fixed factor and $COMT^{158}$ allele status/proline levels as moderating covariate.
Finally, to obtain an integrative model acknowledging the influence of all identified factors on severity of social behavioral symptoms, a backward regression analysis was performed.

Results

Severity of autism symptoms was correlated with COMT status (r=-.345, p=.013 1-tailed) indicating that the $COMT^{MET}$ allele was associated with more severe symptoms, but not with proline level (p=.475). However, the moderated regression model was significant [$F(3,35)$=4.375, p=.010] which revealed a COMT*proline interaction for the (total) severity score (Table 2), indicating that higher problem scores were only seen in individuals with the $COMT^{MET}$ allele who also showed high proline levels (Figure 1). Moderation analyses with the three autism domains revealed comparable COMT*proline interactions for the domains reciprocal social interaction (p=.009) and communication impairment (p=.049), while the effect was not significant for the domain of repetitive and stereotyped behaviors (p=.510).

Table 2 ***Moderation analysis with COMT status and severity of autism symptomatology for testing COMT*Proline interaction.***

Criterion variable	*Predictor/covariate*	*F(df)*	R^2	*β*	*p*
Autism severity (total)	COMT status	*4.375 (3,35)*	.273	-.369	.015
	Proline			.085	.577
	COMT*Proline			-.367	.020

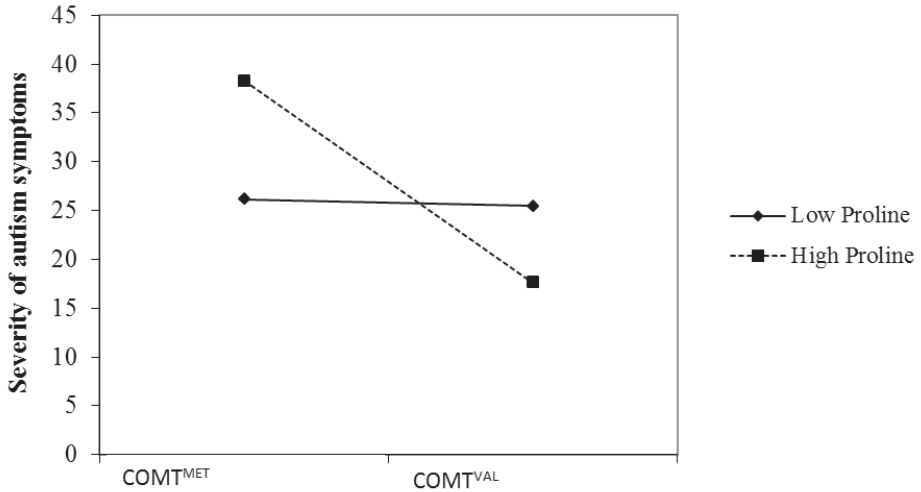

Figure 1 **Interaction of COMT*Proline for severity of total severity autism symptomatology.**

Speed of face recognition and accuracy of facial emotion recognition were correlated with the total severity score (Supplemental Table 1), with poorer quality of social cognition in individuals with more severe social behavioral problems. Using COMT status as covariate, the correlation remained significant for facial emotion recognition, however the relation between face recognition and total severity was no longer significant (Supplemental Table 1). This suggests that poorer quality of emotion recognition is associated with more severe social behavioral problems, independent of COMT genotype.

Regarding face recognition, the COMT*proline interaction resulted in a non-significant model (p=.266). However, a moderated regression analyses revealed a significant interaction between COMT status and face recognition [F(3,34)=4.517, p=.009] (Table 3), indicating that the association of slower face recognition with more severe symptoms holds only for individuals with the $COMT^{MET}$ allele (Figure 2), while no interaction effects with proline were found.

Table 3 ***Moderation analysis with Face recognition and severity of autism symptomatology for testing COMT*Face recognition interaction.***

Criterion variable	*Predictor/covariate*	*F(df)*	R^2	*β*	*p*
Autism severity (total)	COMT status	4.517 (3,34)	.285	-.451	.007
	Face Recognition			.021	.901
	COMT*Face Recognition			-.326	.048

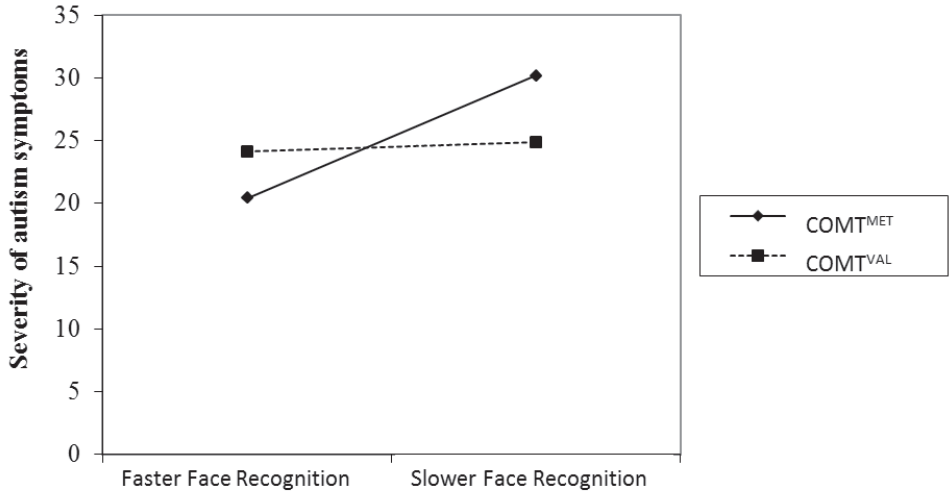

Figure 2 **Interaction of speed of Face Recognition* COMT for severity of total severity autism symptomatology.**

A final multiple regression analysis, attempting to integrate the previous findings, resulted in a significant model [F(4,28)=6.765, *p*=.001], explaining 49.1 % of the variance in severity of social behavioral problems, using COMT status, accuracy of positive emotion recognition, the COMT*proline interaction and the COMT*Face recognition interaction as contributing predictors.
The $COMT^{MET}$ variant was associated with more severe problems, and this association was strongest for those individuals with higher proline levels. Accuracy of positive emotion recognition independent of COMT status and quality of face recognition were associated with more severe problems. For face recognition this association only existed in those individuals with the $COMT^{MET}$ variant (Table 4).

Table 4 ***Multiple regression model (backward): predictors of autism symptom severity.***

Criterion variable	*Predictor/covariate*	*F(df)*	R^2	*β*	*p*
Autism severity (total)	COMT status	6.765 (4,28)	.491	-.405	.007
	Emotion Recognition[1]			.295	.045
	COMT*Proline			-.365	.016
	COMT*Face Recognition			-.271	.062

[1] Accuracy of recognition of positive emotions

Discussion

The influence of the COMT^{158} genotype on variable penetrance of social deficits as well as the possible epistatic interaction of COMT^{158} genotype and plasma proline level were examined in 45 participants with 22q11DS. Outcomes revealed both a main effect of COMT^{158}genotype on severity of social behavioral problems and an interaction between the COMT genotype and proline levels. Individuals with the COMT^{MET} genotype and high proline levels were more likely to present with severe social behavioral problems. In participants with the COMT^{MET} variant poorer quality of face recognition appeared to be associated with more severe social behavioral problems while for individuals with the COMT^{VAL} variant the relation between quality of face recognition and severity of those problems was not present. Poorer quality of emotion recognition, however, was associated with more severe social behavioral problems, independent of COMT^{158} genotype and plasma proline level. An integrative regression model showed that COMT^{158} genotype and its interaction with both proline and quality of face recognition, together with quality of facial emotion recognition accounted for almost 50% of the variance in social behavioral problems.

Although these outcomes need to be interpreted with some caution given the relative small sample size of the study, these findings add to the growing body of research investigating the phenotypic variability in CNVs such as 22q11DS. Elucidating which factors modulate the risk of social cognitive and behavioral problems in 22q11DS may improve our understanding of mechanisms involved in the variable penetrance of phenotypes observed in many CNVs (Jonas *et al.* 2014; Vorstman *et al.* 2013). Therefore, ideally our findings should not only be replicated in a larger sample of 22q11DS patients, but also in carriers of other pathogenic CNVs.

One of the potential mechanism suggested to influence the clinical heterogeneity of 22q11DS are epistatic interactions (Jonas *et al.* 2014; Paterlini *et al.* 2005; Raux *et al.* 2007). Here we have investigated the interaction between COMT^{158} genotypes and plasma proline levels. Our finding that more severe symptomatology in individuals with the COMT^{MET} allele was associated with higher proline levels is in line with the interaction between the COMT and PRODH gene found by Radoeva *et al.* (2014). Additionally, findings suggest that elevated plasma proline levels combined with the COMT^{158} genotype, may have use as a biomarker for the risk of psychopathology

(Raux *et al.* 2007), including – as we show here- vulnerability to autism symptoms, in individuals with 22q11DS.
The results are in line with reports of increased vulnerability to psychiatric disorders in individuals with the $COMT^{MET}$ variant and a negative effect of high proline levels on cognitive and behavioral outcomes in individuals with this variant (Gothelf *et al.* 2007; Lachman *et al.* 1996; Magnee *et al.* 2011; Radoeva *et al.* 2014). Based on research thus far it seems justified to conclude that the co-occurrence of high proline levels and decreased capacity to break down dopamine as a result of carrying the $COMT^{MET}$ variant is associated with unfavorable cognitive and behavioral outcomes in 22q11DS. Our findings add to our understanding of the variable penetrance of cognitive and behavioral phenotypes in individuals with 22q11DS. The impact of the COMT genotype and variations in PRODH (or in their primary downstream effect on plasma proline) shows how variation, other than the deletion itself, can modulate the phenotypic outcome.

Conclusion

Patients with 22q11DS are at increased risk for a range of pathological outcomes, of which several are brain-related. As is the case in most pathogenic CNVs, the penetrance of these phenotypes is highly variable while the underlying mechanisms are poorly understood.
22q11DS, given its high occurrence in the population - i.e. relative to other CNVs- provides a model to examine the mechanisms contributing to variable penetrance. Against this background the reported epistatic interaction between the $COMT^{158}$ genotype and proline on the penetrance of social deficits within 22q11DS, provides valuable insight. We emphasize the importance of investigating these mechanisms in larger 22q11DS samples as well as in patients with other CNVs. Increasing the knowledge about the phenotypic pathway of the different CNVs and their developmental outcomes enables parents and clinicians to meet the challenges of these CNVs and helps to develop early interventions and improve developmental perspectives.

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Supplemental Table 1 ***Pearson- and partial correlations between social cognition and severity of autism symptomatology controlling for COMT status, and Proline levels, respectively.***

		ASD total	COMT status	Proline	FSIQ
Pearson Correlations					
Face Recognition	Reaction Time	.270*	-.300*	.156	-.195
	Accuracy	-.002	.193	.218	-.189
Emotion Recognition (positive)	Reaction Time	.165	-.086	.034	-.289*
	Accuracy	.310*	.053	.095	-.268*
Emotion Recognition (negative)	Reaction Time	.078	-.326*	.123	-.128
	Accuracy	.359**	-.080	.198	-.523**
ASD total		-	-.345**	.010	-.259*
Partial correlations, controlling for COMT status					
Face Recognition	Reaction Time	.186			
	Accuracy	.069			
Emotion Recognition (positive)	Reaction Time	.145			
	Accuracy	.350*			
Emotion Recognition (negative)	Reaction Time	-.039			
	Accuracy	.354*			

**Correlation is significant at the 0.01 level (1-tailed),*Correlation is significant at the 0.05 level (1-tailed)

Chapter 6

Summary and discussion

Many children with 22q11DS experience cognitive and behavioral problems consistent with the two major neurodevelopmental disorders ASD and ADHD (Schneider *et al.* 2014a; Baker & Vorstman 2012; Green *et al.* 2009; Niklasson *et al.* 2009). The studies described in this thesis aimed to provide a better understanding of the mechanisms that result in vulnerability to autism and ADHD symptomatology in individuals with 22q11DS. For this purpose neurocognitive functioning was assessed in a sample of 102 children and adolescents (aged 9-18.5 years) with 22q11DS. Neurocognitive functions can be seen as the expression of the functioning of the brain at an endophenotypic level, by which the association between the genotype (genetic syndrome) and the phenotype (autism and ADHD symptomatology) is mediated. The objective of the current thesis was to investigate the association between neurocognitive functioning and severity of autism and ADHD symptomatology in individuals with 22q11DS. Insight into this association in this specific population is relevant for our understanding of the different trajectories in neurodevelopmental disorders like ASD and ADHD. This knowledge can be used as a starting point for the development and adjustment of preventive interventions and for treatments of children and adolescents with 22q11DS who are at risk of these developmental problems.

Main findings

Neurocognitive functioning in 22q11DS

The studies presented in this thesis provide a profile of neurocognitive strengths and weaknesses that can be viewed as the cognitive phenotype of children and adolescents with 22q11DS. The intelligence profile that was presented in the first study **(chapter 2)** emphasizes the relevance of investigating subdomains of intelligence. When looking at cognitive strengths and weaknesses in our sample we found processing speed and short term attention and memory as relative strengths, which is in line with other studies (Duijff *et al.* 2012; Niklasson & Gillberg 2010). Relative weaknesses in the intelligence profile of children and adolescents with 22q11DS were perceptual organization, sustained attention or concentration, vocabulary, and long term memory. These specific cognitive strengths and weaknesses emphasize the importance of focusing on multiple and more detailed levels of cognitive functioning in this population when evaluating the developmental impact of the syndrome.

Since processing speed proved to be a relative strength in our first study, the outcome that the majority of deficits in executive functioning (EF) were found in accuracy and not in reaction time was a consistent result **(chapter 3)**. Research on EF in children and adolescents with 22q11DS presents consistent evidence for problems in cognitive

control as reflected by the deficits in accuracy of EF (Gur *et al.* 2014; Campbell *et al.* 2010). By evaluating a wide range of EF our study provides a profile of the major strengths and weaknesses in EF in children and adolescents with 22q11DS. The profile revealed that this group failed to perform accurately on tasks that demand cognitive flexibility, resistance against distraction, inhibition and sufficient working memory capacity. We also saw that our group experienced difficulties with planning and sustained attention, from which we conclude that severe impairments in EF form a major characteristic of the neurocognitive phenotype of the syndrome. Since in our study age was associated with poorer quality of EF, and other studies also found similar differences in degree of impairments at different ages, it seems very important to monitor the cognitive development of individuals with 22q11DS and its impact on the developmental outcome of this syndrome.
Social cognition as underlying mechanism of social functioning is an important element for understanding the behavioral disturbances in the social domain that are often found in 22q11DS. We found impairments in both face and facial emotion recognition in our sample with 22q11DS compared to the norm **(chapter 4).** Recognition of positive emotions was relatively less impaired as compared to the recognition of negative emotions. We further observed that quality of abstract visual information processing was also impaired, especially the processing of more complex, featural (nonsocial), information. These findings suggest that children and adolescents with the syndrome experience difficulties in the processing of complex abstract and social visual information. Further analyses gave reason to believe that the deficit in social information processing may at least partly originate from a general impairment in the processing of visuospatial information.

Associations with autism and ADHD symptomatology

The central aim of the research in this thesis was to discover possible mediating mechanisms that are associated with the vulnerability to ASD and ADHD symptomatology in children and adolescents with 22q11DS. Therefore, associations between the profiles of neurocognitive functioning and severity of ASD and ADHD symptomatology, as evaluated in this study, were examined. We first started by looking at differences in intelligence profiles between subgroups with and without symptoms of ADHD and ASD. However, these profiles did not differentiate between participants with and without ASD and/or ADHD. This raises the question whether looking at subgroups based on diagnostic criteria is sensitive enough in this population. It is a clinical reality that children with 22q11DS do have an increased vulnerability to both ASD and ADHD symptomatology. This can be seen as a first argument to investigate both symptom domains instead of following the DSM-IV criteria that, at least formally, do not allow a concurrent diagnosis of both ADHD and ASD in the same individual. Secondly, looking at symptoms that are severe enough to be categorized as a problem score, based on the cut-off criteria of questionnaires or diagnostic criteria, may also not be the most optimal strategy, since a lot of these children experience problems in those domains even when these problems are not severe enough to yield scores in the clinical range.
To overcome these restraints of diagnostic criteria, we decided to use continuous measures of severity on both the ASD and ADHD symptom domains. On the

subdomains of intelligence we found poorer performances on tasks that require perceptual motor integration, visual information processing, comprehension or verbal expression to be associated with more severe ASD symptomatology **(chapter 2)**. Our second study demonstrated impairments in cognitive flexibility, inhibition and distractibility to be associated with more severe ASD symptoms **(chapter 3)**.
Regarding social cognition, poorer accuracy of emotion recognition was associated with more severe ASD symptoms. This association could not be explained by the poorer quality of processing of abstract visual information **(chapter 4)**.
Weaker quality of visuospatial information processing and poorer numerical reasoning, concentration and attention were associated with more severe ADHD symptomatology **(chapter 2)**. More difficulties with sustained attention and a higher distractibility were also associated with more severe ADHD symptomatology **(chapter 3)**. Poorer accuracy of emotion recognition was found to be associated with more severe ADHD symptomatology. However, when using quality of featural processing as a covariate, this association between emotion processing and ADHD symptomatology no longer existed **(chapter 4)**. This could indicate that poorer emotion recognition in children with more severe ADHD symptoms is at least partly explained by poorer visual information processing strategies.
The associations between neurocognitive functioning and ASD and ADHD symptomatology in our sample differs from those in other clinical groups with ASD and ADHD without 22q11DS. This suggests that in the 22q11DS population specific mechanisms are involved in the development and expression of ASD and ADHD symptoms as compared to idiopathic, and therefore highly heterogeneous, ASD and ADHD populations.

Variability in expression of social deficits

In the last chapter we investigated the influence of two additional factors as possible modulating mechanisms of the high vulnerability to social cognitive and behavioral deficits in the 22q11DS population **(chapter 5)**. The contribution of the genotype of the remaining allele of COMT and plasma levels of the amino acid proline to the variability in expression of social deficits was examined. This study demonstrated that individuals with the $COMT^{MET}$ genotype who also displayed high plasma proline levels presented with more severe social behavioral problems. Additionally, in individuals with the $COMT^{MET}$ variant poorer quality of face recognition was associated with more severe social behavioral problems, while in individuals with the $COMT^{VAL}$ variant this association was absent. Lastly, the association between poorer quality of emotion recognition and severity of social behavioral problems that was described in chapter 4 appeared to be independent from $COMT^{158}$ genotype and plasma proline level.

Implications

The findings of this thesis contribute to our understanding of the mediating role of neurocognitive dysfunctions in the development of social and behavioral problems in 22q11DS. From the evidence presented in these studies we concluded that children

and adolescents with this syndrome present with specific cognitive and behavioral phenotypes. The associations between both phenotypes suggest the involvement of a developmental pathway in this syndrome that can be better identified as compared to the diversity of pathway's in the idiopathic ASD and ADHD populations which are, by definition, genetically more heterogeneous. This implies that in children and adolescents with 22q11DS, partly different neurocognitive deficits are associated with the expression and variability of social behavioral problems as compared to children from heterogeneous ASD and ADHD populations. These findings support the idea of different pathways leading to the social behavioral problems reported in developmental disorders (de Zeeuw *et al.* 2012; Durston *et al.* 2011). Based on the outcomes of our last study it can be concluded that differences in developmental pathways are the result of a specific genetic etiology (Bruining et al. 2010). These differences are not only explained by the 22q11.2 deletion itself since variations in COMT gene expression and plasma proline level also play a role in the phenotypic outcome.

Clinical implications

In connection with the existing literature, the neurocognitive profiles described in this thesis emphasize the cognitive strengths and vulnerabilities in children and adolescents with 22q11DS aged 9 – 18.5 years. The results stress the importance to monitor the cognitive development of these children over time, in particular since longitudinal studies report a decline in cognitive functioning somewhere between the age of 7.5 and 15 in children with 22q11DS, with not all domains of functioning equally affected (Duijff et al. 2013; Antshel et al. 2010; Gothelf et al. 2007). How and to what extent the decline in cognitive functioning has an impact on developmental trajectories is not clear yet. Literature and our study suggest that the $COMT^{MET}$ variant might be a risk factor of impairments in cognitive functioning and a unfavorable behavioral outcome (Radoeva et al. 2014; Magnee et al. 2011; Antshel et al. 2010; Gothelf et al. 2007; Lachman et al. 1996;).

Due to their relatively spared processing speed, short attention and memory, there is a risk of overestimating the cognitive capacities of these children. A child with 22q11DS may be seen to keep pace with the normal working tempo in classroom settings. Because of the cognitive difficulties it experiences however, this pace is mostly kept at the cost of quality of cognitive control. Since some of the cognitive dysfunctions identified in this developmental period are also associated with the severity of the social behavioral problems, an attentive focus on their cognitive abilities should be incorporated in protocols for preventive interventions, treatment and care. In view of this recommendation, it is interesting to note that a preliminary study already reported improvement in cognitive skills in adolescents with 22q11DS after following a cognitive remediation program (Harrell *et al.* 2013).

The observed difficulties with visual information processing and in particular with the processing of social relevant information provide important targets for clinical care of these children and adolescents. Especially in this age period (9-18.5 years) these impairments have a large impact on daily functioning. In their interaction with peers and others it is expected that young adolescents are capable of fast perceiving and interpreting visual and social stimuli. Non-verbal communication becomes

increasingly important and their impairment in both processing of abstract and social visual information may hamper social interaction with their peers. It is therefore important to monitor the social development in this age period and to be alert for signs of social exclusion, mood and anxiety problems as a consequence of these impairments.

Scientific implications and directions for future research

The findings presented in this thesis contribute to our understanding of the neurocognitive functioning of children and adolescents with 22q11DS, in particular by adding evidence about associations between quality of neurocognitive skills and severity of autism and ADHD symptomatology. Because of the cross-sectional design of the study we could not look at causal relations between the cognitive and behavioral outcomes. This is only possible by developing longitudinal study designs. For the interpretation and application of our findings in research and clinical settings it is also important to be aware of other limitations. First, although our total sample of *N*=102 is relatively large, the sample size varies between the different studies. This complicates the generalization of findings, especially for the studies that include social cognition (*N*=45). Second, the age range (9-18.5 years) was relatively large and included the transition from childhood into adolescence, the sample being too small to look at the two developmental periods separately or to compare them. Our studies provide important new insights in the associations between the neurocognitive and behavioral phenotype within the syndrome. Because our data consisted of only one time-point we would recommend to investigate longitudinal changes in cognition and its associations with behavioral outcomes later in life. Lastly, the absence of a control group can also be seen as a limitation although it is difficult to determine what would constitute a suitable control group.

The results presented in this thesis not only contribute to our knowledge about the cognitive and behavioral phenotype in 22q11DS, but may also help to understand the developmental pathways in ASD and ADHD. Additionally, our results on the role of genetic variation in the expression of cognitive and behavioral outcomes adds to our understanding of variability in expression of phenotypes in copy number variants (CNVs).

Since cognitive functioning is one of the mediating factors of variability in adaptive functioning of adults with 22q11DS, more insight into how cognitive functioning interacts with behavioral functioning during development may contribute to better adaptive skills later in life (Butcher *et al.* 2012; Schneider *et al.* 2014a). The association that was found between neurocognitive functioning and severity of the observed social behavioral problems explains some of the variability in the cognitive and behavioral phenotype of 22q11DS. Specific deficits in cognitive functioning are associated with more severe behavioral problems and this finding emphasizes the importance to further investigate the influence of cognitive deficits on the variability in phenotypic expressions. Thus far, research focused primarily on the risk of schizophrenia spectrum disorders within the syndrome. Studies have shown an association between impaired cognitive functioning and the presence of (premorbid) schizophrenic symptomatology (Antshel *et al.* 2010; Hooper *et al.* 2013; Schneider *et al.* 2014a; Schneider *et al.* 2014b). However, our findings suggest that it is similarly

important to monitor the cognitive development of children and adolescents with this syndrome in light of social behavioral problems that are part of developmental disorders. It is also important to take other factors into account that possibly influence the associations between neurocognitive functioning and social and behavioral outcomes in later life. For example medical complications, medication effects, interventions and education. Including these factors in the replication and further specification of the demonstrated associations is recommended, especially in longitudinal samples, to provide a better view of the developmental perspective of children and adolescents with 22q11DS.

A second implication concerns the differences in associations between neurocognitive functioning and severity of ASD and ADHD symptomatology as compared to findings in idiopathic groups with ASD and ADHD without 22q11DS. Our results suggest that those children with 22q11DS and ASD or ADHD symptomatology represent a genetic subgroup within the heterogeneous ASD and ADHD populations explaining some of the genetic variation within both developmental disorders (Vorstman & Ophoff 2013; Bruining *et al.* 2014). There might be specific neurocognitive pathways leading to ASD and ADHD symptomatology in individuals with 22q11DS that differ from pathways found in more heterogeneous groups (Durston *et al.* 2011; de Zeeuw *et al.* 2012), although these differences might also be (partly) explained by the previously mentioned factors influencing the association between the neurocognitive and behavioral phenotype in 22q11DS.

Lastly, our research contributes to our understanding of the role of CNVs in the variability of phenotypes of different genetic disorders. CNVs are associated with several neurodevelopmental disorders including autism and ADHD (Moreno-De-Luca & Cubells 2011; Grayton *et al.* 2012). Using 22q11DS as a model of how genetic, cognitive and behavioral factors are influenced by a specific CNV improves our knowledge about the mechanisms that causes variability in expression of phenotypes observed in many CNVs. The interactive effect of $COMT^{158}$ genotypes and plasma proline level on the cognitive and behavioral phenotype in 22q11DS described in **chapter 5** shows us how variation in genes within this specific CNV may be associated with the risk for psychiatric disorders.

In conclusion, this thesis shows the importance of assessing neurocognitive profiles in 22q11DS. Children and adolescents with the syndrome present with severe impairments on various domains of neurocognitive functioning. Some of these impairments are associated with the variable expression of social behavioral problems within the syndrome, underlining the importance of monitoring the cognitive development within this population. For clinical practice and future research it is important to be aware of the role of both genetic factors and neurocognitive functioning in the presence and severity of behavioral problems in 22q11DS and other CNVs. A better understanding of the mechanisms involved in the variable expression of phenotypes will facilitate improvement of clinical care and ultimately lead to a better prediction of developmental outcomes.

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Nederlandse Samenvatting
(Summary in Dutch)

Introductie

Neurobiologische ontwikkelingsstoornissen ontstaan vaak al vroeg in de ontwikkeling van een kind. Genetische factoren hebben, in interactie met de omgeving, invloed op de oorsprong, het beloop en de uitingsvormen van deze stoornissen (American Psychiatric Association. 2013). Ten aanzien van een aantal van deze stoornissen bestaat het idee dat een deel van hun genetische basis gedeeld is. Voorbeeld hiervan zijn de autisme spectrum stoornissen (autism spectrum disorder, ASD) en aandachtsdeficiëntie-/hyperactiviteitsstoornis (attention-deficit/hyperactivity disorder, ADHD) (Rommelse *et al.* 2010; American Psychiatric Association. 2013; Posthuma and Polderman 2013; McCarthy *et al.* 2014). Tot de kernproblemen van ASD behoren de moeite die wordt ervaren met sociale communicatie en interactie en het beperkte en repetitieve repertoire van gedragspatronen, interesses en activiteiten. Beperkingen in het dagelijks functioneren door onoplettendheid, hyperactiviteit en/of impulsiviteit zijn de belangrijkste kenmerken van ADHD (American Psychiatric Association. 2013). De veronderstelde overlap in genetische oorsprong tussen ASD en ADHD geeft aanleiding om de aanwezigheid en ernst van symptomen behorende bij deze stoornissen binnen een genetisch syndroom te bestuderen en zodoende de kennis met betrekking tot de onderliggende mechanismen van deze stoornissen te vergroten (Rutter 1997; Scourfield *et al.* 1999).

Het 22q11.2 deletie syndroom (22q11DS) is een duidelijk voorbeeld van een genetische stoornis waarbij symptomen van ASD en ADHD onderdeel zijn van het gedragsfenotype. Het syndroom dat ook wel bekend is onder de namen velo-cardio-facial syndroom (VCFS) en DiGeorge syndroom komt voor bij ongeveer 1 op de 2.000-4.000 levend geborenen. Dit betekent dat in Nederland elk jaar ongeveer 50 kinderen (ongeveer evenveel jongens als meisjes) met dit syndroom worden geboren (Devriendt *et al.* 1998; Oskarsdottir *et al.* 2004; Shprintzen 2008). Het syndroom is autosomaal overdraagbaar wat betekent dat indien een van de ouders het syndroom heeft, de kans 50% is dat een kind het erft. In 90% van de gevallen echter gaat het om een nieuwe mutatie waarbij beide ouders geen drager zijn van het genetisch defect (Shprintzen 2008). Het fenotype van het syndroom is variabel met verschillende fysieke kenmerken. Zo is er vaak sprake van een aangeboren hartafwijking, problemen met het immuunsysteem, afwijkingen aan het gehemelte en hypernasale spraak (Swillen *et al.* 2000; Bassett *et al.* 2011; Cancrini *et al.* 2014). Ook zijn er vaak karakteristieke gelaatskenmerken zoals amandelvormige ogen, een brede neusbrug, kleine en laag ingeplante oren en dunne vingers (Bassett *et al.* 2011). Het neurocognitieve fenotype wordt gekenmerkt door een vertraagde motorische ontwikkeling, spraak-, taal- en leerproblemen. Het cognitieve profiel van mensen met 22q11DS laat veel individuele verschillen zien en kan variëren van een beneden

gemiddeld intelligentieniveau tot een milde of ernstige verstandelijke beperking (De Smedt *et al.* 2007; Niklasson & Gillberg 2010; Philip & Bassett 2011;Duijff *et al.* 2012). Naast ASD en ADHD komen angststoornissen, oppositionele gedragsstoornissen en stemmingsstoornissen veel voor binnen de 22q11DS populatie en ontwikkelt ongeveer 25% van de adolescenten en volwassenen met dit syndroom schizofrenie (Murphy *et al.* 1999; Jolin *et al.* 2009; Baker & Vorstman 2012; Jonas *et al.* 2014; Schneider *et al.* 2014;).

Doel en opzet van het onderzoek

De studies beschreven in dit proefschrift hadden tot doel om beter te begrijpen welke mechanismen een rol spelen bij de kwetsbaarheid die kinderen en adolescenten met 22q11DS laten zien voor het ontwikkelen van ASD en ADHD symptomen. Daarbij lag de focus op het onderzoeken van de relatie tussen de kwaliteit van het neurocognitief functioneren en de ernst van de aanwezige ASD en ADHD symptomen. Neurocognitieve functies reflecteren de complexe mechanismen van onze hersenen en zijn gerelateerd aan specifieke gebieden of netwerken binnen ons brein. Deze functies worden gebruikt om informatie te verwerken en ons gedrag aan te sturen (Swaab *et al.* 2011). Het onderzoeken van de relatie tussen deze neurocognitieve functies en de sociale en gedragsproblemen van kinderen en adolescenten met 22q11DS draagt bij aan het beantwoorden van de vraag of de relatie tussen een genetische component (in dit geval 22q11DS) en de ontwikkeling van problemen op gedragsniveau verklaard kan worden vanuit de mediërende rol van neurocognitieve functies.

De beschreven studies in dit proefschrift vormen een onderdeel van een nationaal onderzoek naar 22q11DS vanuit het Universitair Medisch Centrum Utrecht (UMCU). De 102 kinderen en adolescenten die hebben meegedaan aan ons onderzoek waren 9-18,5 jaar oud op het moment dat zij onderzocht werden. Voor de verschillende studies werden de volgende doelen geformuleerd:

1) Het vergroten van de kennis met betrekking tot intelligentieprofielen van kinderen en adolescenten met 22q11DS en het onderzoeken of er een relatie bestaat tussen de sterktes en zwaktes binnen deze profielen en de ernst van aanwezige ASD en ADHD symptomen (Hoofdstuk 2).
2) Onderzoeken of er een specifiek profiel van executieve functies (EF) bestaat binnen de 22q11DS populatie en nagaan of de kwaliteit van deze executieve functies samenhangt met de sociale en gedragsproblemen die onderdeel zijn van de ontwikkelingsstoornissen ASD en ADHD (Hoofdstuk 3).
3) Onderzoeken van de relatie tussen de kwaliteit van sociaal cognitieve functies en de ernst van de sociale problemen van kinderen en adolescenten met 22q11DS. In het bijzonder een antwoord vinden op de vraag of algemene problemen met visuele informatieverwerking samenhangen met de kwaliteit van vaardigheden op het gebied van gezichtsherkenning en emotieherkenning en of deze algemene problemen de relatie tussen de sociaal cognitieve vaardigheden en de ernst van de ASD en ADHD symptomen mede beïnvloeden (Hoofdstuk 4).

4) Eerdere studies hebben aangetoond dat het COMT[158] genotype en de plasma proline waarden in het bloed samen hangen met sociale cognitie in verschillende klinische populaties. De rol van deze factoren in de kwetsbaarheid voor sociaal cognitieve en gedragsproblemen in de 22q11DS populatie is nog niet bekend en vormde het onderwerp van de laatste studie (Hoofdstuk 5).

Onderzoeksbevindingen

Intelligentie en de relatie met autisme en ADHD symptomen binnen het 22q11DS

De bevindingen in hoofdstuk 2 beschrijven de cognitieve sterktes en zwaktes van kinderen en adolescenten met 22q11DS zoals die zich in het intelligentieprofiel laten zien. Verwerkingssnelheid, korte termijn geheugen en alertheid bleken relatief sterke vaardigheden binnen het intelligentieprofiel. Deze bevinding sluit aan bij eerdere studies (Niklasson & Gillberg 2010; Duijff *et al.* 2012). Perceptuele organisatie, volgehouden aandacht, concentratie, woordenschat en lange termijn geheugen zijn relatief minder sterk ontwikkeld binnen onze onderzoeksgroep. Dit profiel van relatieve sterktes en zwaktes in het cognitief functioneren geeft aan dat het belangrijk is om op een meer gedetailleerde manier naar cognitie te kijken om de impact van het syndroom op de ontwikkeling beter te kunnen onderzoeken.
Wanneer we de intelligentieprofielen van kinderen met en zonder ASD en/of ADHD kenmerken vergeleken vonden we geen verschillen tussen deze groepen. Dit roept de vraag op of het onderscheid op basis van diagnostische criteria zinvol is als het gaat om het begrijpen van cognitieve mechanismen binnen 22q11DS. Het is een klinische realiteit dat kinderen met dit syndroom een verhoogde kwetsbaarheid hebben voor zowel ASD als ADHD symptomatologie. Op basis van dit gegeven lijkt het raadzaam om bij het individuele kind met 22q11DS beide symptoomdomeinen in kaart te brengen om de impact van de stoornis op het functioneren te beschrijven, hoewel het volgens de criteria van het Amerikaanse classificatiesysteem DSM niet gebruikelijk is om zowel ADHD als ASD te diagnosticeren in één individu (American Psychiatric Association. 2013). Het in kaart brengen van de ernst van de symptomen, ongeacht of deze tot een diagnostische classificatie zouden leiden, heeft als voordeel dat men recht doet aan de situatie dat veel kinderen met 22q11DS problemen ervaren binnen beide domeinen, maar dat deze problemen vaak niet ernstig genoeg zijn om binnen de klinische range te vallen op basis van de cutt-off criteria van vragenlijsten en diagnostische criteria. Om zicht te krijgen op de impact van de aandoening voor het dagelijks functioneren hebben wij er voor gekozen om gebruik te maken van continue maten voor het in kaart brengen van de ernst van ASD en ADHD symptomen.
Wanneer we keken naar de relaties tussen de prestaties op de verschillende intelligentiedomeinen en de ernst van de ASD en ADHD symptomen, bleken ernstigere ASD symptomen samen te hangen met zwakkere prestaties van de kinderen en adolescenten wanneer een beroep werd gedaan op visueel motorische integratie, visuele informatieverwerking, taalbegrip en verbale expressie. Problemen met

volgehouden aandacht en een snellere afleidbaarheid bleken samen te hangen met meer ADHD symptomen.

Executief functioneren en autisme en ADHD symptomen binnen het 22q11DS

Hoofdstuk 3 in deze studie laat opnieuw zien dat binnen het 22q11DS ernstige beperkingen bestaan in EF waarbij kinderen en adolescenten veel problemen ervaren op het gebied van cognitieve flexibiliteit, inhibitie, volgehouden aandacht, afleidbaarheid, werkgeheugen en planning, met name wat betreft nauwkeurigheid in prestaties. Daarnaast bleken een zwakke cognitieve flexibiliteit en inhibitie en een hoge mate van afleidbaarheid gerelateerd aan ernstiger ASD symptomen. Ook hingen een slechte volgehouden aandacht en een hoge afleidbaarheid samen met ernstiger ADHD symptomen.
Binnen onze studie bleek de impact van de beperkingen in EF verschillend te zijn per leeftijd. Ook in eerdere studies is al aangetoond dat er verschillen zijn in de mate van beperking in EF van kinderen en adolescenten met 22q11DS op verschillende leeftijden (Anstel *et al.* 2010; Stoddard *et al.* 2011). Het is dan ook in het individuele geval van belang om de cognitieve ontwikkeling nauwkeurig te volgen alsmede de impact van deze cognitieve ontwikkeling op de ontwikkelingsperspectieven van individuen met dit syndroom.

Sociale cognitie en autisme en ADHD symptomen binnen het 22q11DS

Sociale cognitie als onderliggend mechanisme van sociaal functioneren is een belangrijke factor in het begrijpen van de gedragsproblemen binnen het sociale domein welke vaak worden gerapporteerd bij individuen met 22q11DS. Hoofdstuk 4 beschrijft de beperkingen die gevonden werden op het gebied van gezichtsherkenning en het herkennen van emoties. Kinderen en adolescenten met 22q11DS bleken hier meer moeite mee te hebben in vergelijking met hun leeftijdsgenoten. Ze hadden relatief minder moeite met het herkennen van positieve emoties ten opzichte van negatieve emoties. Daarnaast vonden we ook beperkingen in de verwerking van abstracte visueel ruimtelijke informatie, waarbij de deelnemers vooral moeite hadden met het herkennen van subtiele verschillen in patronen. Deze bevindingen duiden erop dat de moeite die deze groep ervaart met het verwerken van sociale informatie mogelijk ten dele verklaard kan worden door een meer algemene beperking in het verwerken van visuele informatie. Ernstiger problemen met het adequaat herkennen van emoties was gerelateerd aan meer ASD en ADHD symptomen. Echter, de relatie tussen kwaliteit van emotieherkenning en ernst van ADHD symptomen werd verklaard door algemene beperkingen in het verwerken van visuele informatie. De relatie met ASD symptomen werd niet verklaard door deze algemene problemen in het verwerken van visuele informatie. Beperkingen in de verwerking van zowel abstracte als sociale visuele informatie lijken dus onderdeel te zijn van het cognitieve fenotype van 22q11DS en ernstiger beperkingen in sociale cognitie zijn te vinden bij kinderen met meer ASD problematiek.

COMT genotype en plasma proline waarden en variabiliteit in sociale problemen

De 22q11.2 deletie heeft invloed op de uiting van verschillende genetische componenten welke zich op dit chromosoom bevinden. Zo is er van het COMT gen maar één allel aanwezig in plaats van zoals gebruikelijke twee en heeft de deletie ook invloed op het functioneren van het PRODH gen. Aangezien beide genen mogelijk van invloed zijn op sociaal cognitieve en gedragsproblemen onderzochten we de invloed van het COMT genotype en de plasma proline waarden (waar het PRODH gen voor decodeert) op de variabiliteit in expressie van deze problemen. De uitkomsten, in hoofdstuk 5 beschreven, laten zien dat individuen waarbij zowel de $COMT^{MET}$ variant en hoge waarden van de plasma proline waarden aanwezig waren meer sociale gedragsproblemen hebben. Daarnaast bleek bij individuen met de $COMT^{MET}$ variant de kwaliteit van gezichtsherkenning samen te hangen met de ernst van de sociale gedragsproblemen, terwijl dat niet het geval was bij individuen met de $COMT^{VAL}$ variant. De relatie tussen emotieherkenning en sociale problemen bleek onafhankelijk te zijn van het COMT genotype en de plasma proline waarden.

Implicaties

De resultaten van de beschreven studies hebben een aantal implicaties voor zowel de klinische praktijk als voor verder wetenschappelijk onderzoek.
Allereerst geven de gevonden neurocognitieve profielen opnieuw aan dat het binnen deze populatie erg belangrijk is om de cognitieve ontwikkeling van kinderen en adolescenten met 22q11DS nauwkeurig te volgen. Studies tot nu toe beschrijven een uniek profiel van neurocognitieve sterktes en zwaktes welke kenmerkend lijkt te zijn voor de 22q11DS populatie (hoofdstuk 2,3,4 ; Gothelf *et al.* 2007; Antshel *et al.* 2010; Duijff *et al.* 2012). De consequenties van deze kenmerkende cognitieve ontwikkeling voor de verdere ontwikkeling en het functioneren in de volwassenheid zijn nog niet duidelijk in kaart gebracht. Longitudinale studies zijn daarvoor noodzakelijk. Voor het dagelijks leven van deze kinderen is het van belang dat ouders en leerkrachten zich bewust zijn van het risico dat bestaat om de cognitieve capaciteiten van deze kinderen te overschatten, vanwege de specifieke kenmerken van het cognitieve profiel. Aangezien verwerkingssnelheid, alertheid en korte termijn geheugen relatief sterk zijn, kan het zo zijn dat deze kinderen het tempo in de klas lijken bij te kunnen houden. Echter dit tempo zal voor deze kinderen al snel ten koste gaan van de kwaliteit van de overige cognitieve functies welke nodig zijn om informatie goed te verwerken en toe te passen. De gevonden relaties tussen beperkingen in het neurocognitief functioneren en de sociale en gedragsproblemen geven aan dat het belangrijk is om bij bestaande en nieuwe behandelingen en interventies het profiel van cognitieve vaardigheden als uitgangspunt te nemen. Deze aanbeveling wordt ondersteund door de bevindingen van een eerdere studie waarin een cognitief interventie bij adolescenten met 22q11DS effectief bleek te zijn (Harrell *et al.* 2013).
Ten tweede vergroten de bevindingen van onze studies het huidige inzicht met betrekking tot de samenhang tussen beperkingen in het neurocognitief functioneren

en de ontwikkeling van sociale en gedragsproblemen bij kinderen en adolescenten met 22q11DS. De gevonden relatie tussen het cognitieve en gedragsfenotype duidt op een unieke ontwikkeling welke specifiek lijkt te zijn voor 22q11DS. Wanneer we de gevonden relatie tussen het neurocognitief functioneren en de ASD en ADHD symptomen vergelijken met bevindingen binnen ASD en ADHD populaties zonder 22q11DS blijken er verschillen te zijn in de gevonden associaties tussen neurocognitieve beperkingen en ASD en ADHD problematiek. Deze bevindingen ondersteunen ideeën in de literatuur over het bestaan van verschillende ontwikkelingspaden welke leiden tot de sociale gedragsproblemen behorende bij beide neurobiologische ontwikkelingsstoornissen (Durston *et al.* 2011; de Zeeuw *et al.* 2012). Belangrijk om hier bij op te merken is dat in onze studie niet gekeken is naar mogelijke factoren die van invloed zijn op de relatie tussen neurocognitieve beperkingen en ASD en ADHD problematiek. Daarbij valt te denken aan medische complicaties, effecten van medicatie, cognitieve en gedragsmatige interventies en onderwijs. De bevindingen van de studie beschreven in hoofdstuk 5 suggereren dat bijkomende genetische factoren ook van invloed zijn op deze verschillende ontwikkelingspaden. Het lijkt erop dat naast de 22q11.2 deletie ook andere genetische variatie, waaronder de expressie van het COMT genotype en de plasma proline waarden, van invloed is op de uitkomsten in de ontwikkeling van cognitieve en gedragsproblemen (hoofdstuk 5; Bruining *et al.* 2010).
Ten slotte is het van belang dat de gevonden relaties tussen neurocognitief functioneren en de aanwezige sociale en gedragsproblemen worden onderzocht in longitudinale studies en studies die zich richten op meerdere aspecten van de voor 22q11DS kenmerkende psychopathologie. Het is bekend dat het cognitief functioneren een van de factoren is welke invloed heeft op het adaptief kunnen functioneren van volwassenen met 22q11DS (Butcher *et al.* 2012; Schneider *et al.* 2014). Het is dan ook van belang om te onderzoeken wat de invloed is van de cognitieve beperkingen die gezien worden bij kinderen en adolescenten met 22q11DS op hun ontwikkeling en kwaliteit van leven als volwassene.

Conclusie

De resultaten van dit proefschrift laten het belang zien van neurocognitieve profielen binnen het onderzoek naar neurobiologische ontwikkelingsstoornissen. De bevindingen tonen aan dat kinderen en adolescenten met 22q11DS ernstige beperkingen laten zien op het gebied van neurocognitief functioneren. Daarbij hangen deze beperkingen ook samen met de variabiliteit in expressie van sociale en gedragsproblemen bij deze kinderen. Het is dus van belang om de neurocognitieve ontwikkeling van individuen met 22q11DS goed te volgen en deze, samen met de invloed van de genetische aspecten van het syndroom, te betrekken bij het onderzoek naar neurobiologische ontwikkelingsstoornissen binnen deze populatie. Het beter begrijpen van de mechanismen die een rol spelen bij het ontstaan, de ernst en de variabiliteit in expressie van sociale en gedragsproblemen kan leiden tot verbetering van de zorg en begeleiding en zal het uiteindelijk ook mogelijk maken om beter te voorspellen wat men kan verwachten van de ontwikkeling van kinderen met een syndroom als 22q11DS.

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Curriculum Vitae

Elske Hidding was born on October 30th of 1987 in Oosterhesselen, The Netherlands. After completing her secondary education (VWO) in 2006 at the Greijdanus College in Zwolle, she studied Clinical Child and Adolescent studies at Leiden University. She obtained her master's degree in 2011 after completing the research master Developmental Psychopathology in Education and Child Studies.
This master included clinical internships at Centrum Autisme in Oegstgeest and the University Outpatient Department (Ambulatorium) in Leiden. She wrote her master's thesis on 'Executive functioning in relation to proactive and reactive aggression in childhood and adolescence'.
She has worked as a junior child psychologist at Centrum Autisme and in 2012 she started her PhD project at the department of Clinical Child and Adolescent Studies of Leiden University. This project, supervised by Prof. Hanna Swaab, Dr. Ir. Leo de Sonneville, Dr. J.A.S. Vorstman, and Prof. Herman van Engeland, resulted in the present thesis.
Besides her work as at the PhD project, she was also appointed as a lecturer at the same department.

List of Publications

Hidding, E., Swaab, H., De Sonneville, L.M.J., Van Engeland, H., Sijmens-Morcus, M. E. J., Klaassen, P. W. J., Duijff, S. N., & Vorstman, J. A. S. (2015). Intellectual functioning in relation to autism and ADHD symptomatology in children and adolescents with 22q11.2 Deletion Syndrome. *Journal of Intellectual Disability Research.* DOI: 10.1111/jir.12187

Hidding E., De Sonneville L.M.J. Van Engeland H., Vorstman J.A.S., Sijmens-Morcus, M.E.J., & Swaab H. Executive function in and its relation to autism and ADHD symptomatology in 22q11 Deletion Syndrome. *Under review*

Hidding E., De Sonneville L.M.J., Van Engeland H., Vorstman J.A.S., & Swaab H. Facial emotion processing and its relation to autism and ADHD symptomatology in 22q11 Deletion Syndrome. *Revised manuscript under review*

Hidding, E., Swaab, H., De Sonneville, L.M.J., Van Engeland, H., & Vorstman, J.A.S. The role of COMT and plasma proline in the variable penetrance of social deficits in 22q11.2 Deletion Syndrome. *Revised manuscript submitted*

Abstracts

Hidding E., Swaab H., Vorstman J.A.S., Van Engeland H., Sijmens-Morcus, M.E.J., Klaassen P.W.J., Duijff S.N., & De Sonneville L.M.J. (2013). Intelligence profiles in children and adolescents with 22q11 Deletion Syndrome with and without psychopathology, International Society for Autism Research (IMFAR), San Sebastian, Spain

Hidding E., De Sonneville L.M.J. Van Engeland H., Vorstman J.A.S. & Swaab H. Executive functioning in relation to autism and ADHD symptomatology in 22q11 Deletion Syndrome, (2014). *Journal of Intellectual Disability Research,* 58: 883–889. doi: 10.1111/jir.12156, The **Society for the Study of Behavioural Phenotypes (SSBP),** New York, US.

Hidding E., De Sonneville L.M.J. Van Engeland H., Vorstman J.A.S. & Swaab H. (2014). Social cognition in relation to autism and ADHD symptomatology in 22q11 Deletion Syndrome, The **Society for the Study of Behavioural Phenotypes (SSBP),** New York, US.